Aus dem Departement für Pferde,
Abteilung Anästhesiologie der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. med. vet. Anton Fürst

Arbeit unter der Leitung von
Prof. Dr. med. vet. M. Paula Larenza,
Matthew J. Ryan Veterinary Hospital, University of Pennsylvania,
Philadelphia, USA

Intraoperative effects and post operative recovery quality after racemic ketamine or S (+) ketamine administered to male dogs undergoing elective neutering surgery.

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vorgelegt von

Boris M. Pfaender

Tierarzt aus
La Neuveville (BE)

Genehmigt auf Antrag von

Prof. Dr. med. vet., PhD Regula Bettschart-Wolfensberger, Referentin
Prof. Dr. med. vet. H.P Nägeli, Korreferent

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Summary

Anaesthesia with S (+) ketamine results in better recoveries in humans, cats and horses than with racemic ketamine at dose rates of only 50-66% of the racemic ketamine.

This prospective, blinded, randomized trial compared anesthesia induction with intravenous S (+) ketamine (2 mg/kg, n= 20) and racemic ketamine (4 mg/kg, n=20) in 40 dogs undergoing castration. Sedation was performed with intramuscular medetomidine and butorphanol in both groups. For analgesia lidocaine was applied locally. Anaesthesia was monitored as under clinical circumstances. Sixty minutes after S (+) or racemic ketamine atipamezole was administered and recovery quality assessed with standardized scores 0, 10, 20, 30, and 60 minutes thereafter.

During the intraoperative phase, three dogs in the S (+) ketamine group needed an extra bolus of intravenous S (+) ketamine to maintain adequate anaesthesia depth. Another two dogs, one in each group, showed spasms immediately post-operatively which needed treatment and were excluded from comparison. Respiration was better maintained with S (+) ketamine. Recovery was not different between the groups.

Half dose of S (+) ketamine provided similar anaesthesia quality, cardiopulmonary function and recovery as racemic ketamine. Only dogs with S (+) ketamine needed redosing to finish the surgery. This might suggest that duration of action of S (+) ketamine is slightly less than that of racemic ketamine, as has been suggested in other species.

Keywords: Dogs, S (+) ketamine, racemic ketamine, anaesthesia induction, recovery

1. Introduction

Ketamine is a non-competitive glutamate N-methyl-D-aspartate (NMDA) receptor antagonist (Anis *et al.*, 1983) used in human and veterinary anaesthesia, which is composed of two optical isomers in a 50:50% racemic mixture (Kharasch and Labroo, 1992; Kharasch *et al.*, 1992). The 2 isomers present in the racemic mixture have different pharmacodynamic and pharmacokinetic properties. The levorotatory enantiomer, S (+) ketamine, has potential advantages as compared to the dextrorotatory enantiomer and to the racemic mixture including: a) a stronger anaesthetic effect (White *et al.*, 1985), b) a quicker recovery from anaesthesia (Doenicke *et al.*, 1992; White et al., 1985), c) reduction of uncontrolled locomotor activity during recovery (White *et al.*, 1985), and d) a more advantageous profile regarding cardiovascular side effects (Graf *et al.*, 1995). This is why the use of S (+) ketamine has gained wide acceptance in human medicine, and much effort are being undertaken to explore more precisely its properties in veterinary medicine.

S (+) ketamine is currently registered in Switzerland for feline use only. The pharmaceutical company Dr. E. Graeub AG is the first company to register the S (+) ketamine under the trade name Keta S ®, ad us vet. In cats undergoing elective orchiectomy, S (+) ketamine provided faster recovery from anaesthesia than racemic ketamine with only 60% of the racemic dose. In this study, postoperative analgesia was considered similar for both formulations. S (+) ketamine has also been successfully used in ASA III cat patients offering a significantly faster recovery as compared to the racemic mixture (Larenza *et al.*, 2008). Post-operative analgesia and recovery quality were judged significantly better in this study as well.

A recent pharmacokinetic study conducted in dogs showed that S (+) ketamine has a higher plasmatic clearance as compared to the R (-) isomer and to the racemic form (Henthorn *et al.*, 1999). That might explain why the anaesthetic recovery is faster in patients anaesthetised with S (+) ketamine. In assays testing the equipotency of different ketamines in dogs, quality and length of anaesthesia were similar after IV injection of 10 mg/kg of racemic ketamine or 6.6 mg/kg of S (+) ketamine (Deleforge *et al.*, 1991). In contrast, another study performed in dogs that received IV racemic ketamine at 9 mg/kg or S (+) ketamine at 6 mg/kg, showed that all anaesthetic periods were longer for dogs treated with racemic ketamine (Duque *et al.*, 2008). In regard to cardiovascular function, previous studies performed in dogs using equivalent doses of racemic or S (+) ketamine did not show differences in the cardiopulmonary effects induced by 30 mg/kg IV of either compound (Muir and Hubbell, 1988). Similarly, comparable electrophysiological effects after 20 mg/kg of either drug were observed and it was concluded that both formulations have very similar effects on cardiac electrical conductivity (Souza *et al.*, 2002).

However, to date there are no clinical reports comparing S (+) and the racemic form of ketamine using equipotent doses in dogs. Previous studies used equivalent doses and results were not conclusive.

Therefore, the present study was designed to compare the anaesthetic properties of two doses of racemic and S (+) ketamine in canine patients undergoing an elective surgical procedure.

2. Literature

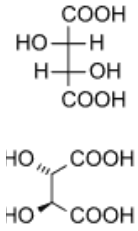
2.1 Isomerism

2.1.1 Discovery of Isomers

In 1812, Jean-Baptiste Biot studied the polarisation of light passing through chemical solutions and presented a comprehensive theory showing how crystalline solids and samples containing an excess of one enantiomer of a chiral molecule rotate the orientation of plane-polarised light. He discovered chirality in 1832 by observing the ability of a tartaric acid solution to rotate polarised light.

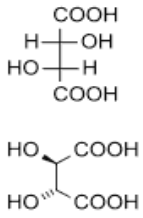
In 1828 Joseph Louis Gay-Lussac coined the name racemic acid (acide racémique, from the Latin *racemus*, for a bunch of grapes) to describe the para-tartaric acid and demonstrated that this racemate had the same chemical composition as tartaric acid.

Louis Pasteur, made a series observations involving solutions of tartaric acid (from the tartar deposits in maturing barrels of wine (Fig. 1) and a second form of this acid, known as para-tartaric acid. Pasteur noted that the two crystals were mirror images of each other. Pasteur manually separated these crystals and noted that the separate solutions, with equivalent concentrations of tartaric acid crystals, rotated in linearly polarised light in equal but opposite directions. In addition, when equal parts of the two tartaric acid solutions were combined, no optical rotation was observed. From this observation, Pasteur inferred that the optically inactive racemic acid was composed of equal amounts of (+) and (−) enantiomers of tartaric acid (Louis Pasteur).



Levotartaric acid

R (−) tartaric acid



Dextrotartaric acid

S (+) tartaric acid

Fig.1: Tartar deposits in maturing barrels of wine and tartaric acid (racemic acid) when in 1:1 ratio, chemical formulas of the 2 compounds

2.1.2 Definitions

Enantiomer is one of two stereoisomers that are non-super imposable mirror images (Fig. 2) of each other. They are compared with one's left and right hands, with the same shape but opposite.

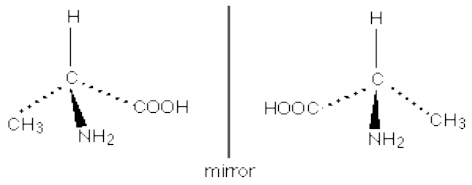


Fig. 2: Example of 2-aminopropanoic acid (alanine)

Stereoisomers are isomeric molecules that have the same molecular formula and sequence of bonded atoms (constitution), but which differ in the three dimensional orientations of their atoms in the space.

Isomers are compounds with the same molecular formula but have different structural formulae.

Racemate or racemic mixture is a compound that has equal amounts of left- and right-handed enantiomers of a chiral molecule.

2.1.3 Structural Isomerism

There are 3 forms of structural isomerism:

Position isomerism

Position isomers have a functional group in a different position on the chain (Fig. 3).

Skeletal isomerism

In skeletal isomerism, or chain isomerism, components of the skeleton are distinctly re-ordered to create different structures. For example (Fig. 3) 3-methylpentane is a chain isomer of 2-methylpentane

Tautomerism

Tautomerism is a special case of structural isomerism. Tautomers are organic compounds that are interconvertible (Fig. 4) by a chemical reaction called tautomerisation. In solutions where tautomerisation is possible, a chemical equilibrium of the tautomers will be reached. The exact ratio of the tautomers depends on several factors, including temperature, solvent, and pH.

Fig. 3: Methylpenthane

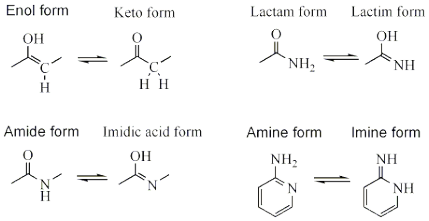


Fig. 4: Tautermer examples

2.1.4 Different types of isomerism

Stereoisomerism can be distinguished in geometrical and optical isomerism.

2.1.4.1 Geometrical isomerism

Also known as cis-trans isomerism geometrical is a form of stereoisomerism. Also known as Z (from the German *zusammen*, together or if they are on opposite sides it is E (from the German *entgegen*, opposite). Dichloroethene illustrate an example for geometrical isomerism (Fig. 5)

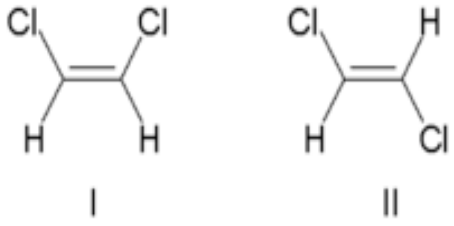


Fig. 5: An example of double bond stereoisomerism is 1,2-dichloroethene, $C_2H_2Cl_2$.Molecule I is Z-1,2-dichloroethene and molecule II is E-1,2-dichloroethene.

2.1.4.2 Optical isomerism

Optical isomers are named like this because of their effect on plane polarised light. Simple substances, which show optical isomerism, exist as two isomers known as enantiomers.

A solution of one enantiomer rotates the plane of polarisation in a clockwise direction. This enantiomer is known as the (+) form or dextrorotatory isomer.

For example, one of the optical isomers (enantiomer) of the amino acid alanine is known as (+) alanine.

A solution of the other enantiomer rotates the plane of polarisation in an anti-clockwise direction. This enantiomer is known as the (-) form or levorotatory isomer. So the other enantiomer of alanine is known as or (-) alanine.

If the solutions are equally concentrated, the amount of rotation caused by the two isomers is exactly the same but in opposite directions.

When optically active substances are made in the lab, they often occur as a 50/50 mixture of the two enantiomers. This is known as a *racemic mixture* or *racemate*. It has no effect on plane-polarised light.

2.1.5 Nomenclature

Two principal systems of nomenclature have been developed to describe the absolute configuration of asymmetric molecules. According to the first convention (Fisher), two reference compounds, the natural levorotatory enantiomer of serine (designated L-serine) and the natural dextrorotatory enantiomer of glyceraldehydes (designated D-glyceraldehyde). On the other hand, a second convention introduced by Cahn et al (1956), is based on a set of rules for assigning an order of decreasing priority (a± d) to each of the substituents attached to the asymmetric carbon, decreasing atomic number being the simplest situation. The molecule is then viewed with the lowest priority group away from the viewer. If the direction of rotation from the highest to the lowest priority group is to the left, the enantiomer can be described as S (sinister) and, if the direction of rotation is to the right, then the molecule is R (rectus) (Landoni *et al.*, 1997; Landoni and Lees, 1996).

2.1.6 Examples from the pharmacology

Many drugs currently used in medical practice are mixtures of enantiomers. Often the two enantiomers differ in their pharmacokinetic and pharmacodynamic properties. Replacing existing racemates with single isomers has resulted in improved safety and/or efficacy profile of various racemates. Some important chiral switches that have yielded

safer alternatives include levosalbutamol, S (+) ketamine, S (+) zopiclone, levocetirizine, S (+) amlodipine, S (+) metoprolol, S (+) omeprazole, S (+) pantoprazole and R (-) ondansetron (Patil and Kothekar, 2006).

2.2 The dissociative anaesthetics drugs

Dissociative anaesthetic drugs are cyclohexanone derivates (Fig. 6) that induce anaesthesia, analgesia, and immobilisation in the treated animal. The term ‘dissociative’ represents the state of the immobilisation induced by cyclohexamines. In this condition, the treated animal is typically unresponsive to stimulation but retains normal pharyngeal and laryngeal reflexes (cataleptic stage). The eyes remain open and may show nystagmic gaze. Palpebral and corneal reflexes are often present. This class of anaesthetics does not produce muscle relaxation. On the contrary, they may induce muscle rigidity or twitching. Dissociative anaesthetics induce analgesia, disorientation, excessive salivation, hypertension or hypotension, hallucinations, vocalisation, and may develop tonic clonic convulsions (Nielsen, 1999).

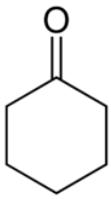


Fig. 6: Cyclohexanone derivate

Cyclohexanone derivates anaesthetics include: phencyclidine, tiletamine and ketamine.

2.2.1 Phencyclidine (PCP)

PCP (Fig. 7) was the first cyclohexanone derivative to be used for animal immobilisation. Originally labelled as CI-395, it was synthesised for use in human anaesthesia in the early 1960s. Because of illicit human use (strong hallucinogenic action) the molecule was abandoned for this purpose, reclassified by the Food and Drug Administration and taken off the market in 1978 (Vollenweider, 2003 and Vollenweider-Scherpenhuyzen, 2003; Nielsen, 1999).

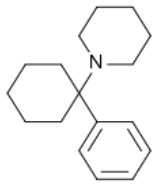


Fig. 7: Phencyclidine (PCP)

2.2.2 Tiletamine (HCl)

Available only in combination with the benzodiazepine, zolazepam, in the commercial form known as Telazol ® and Zoletil ®, Tiletamine (Fig. 8) was synthesised in the 1970s as CI-634. The factors that limit, under practice conditions the use of this combination are the instability of zolazepam once mixed with tiletamine (Nielsen, 1999) and furthermore, the elevated price, compared to racemic ketamine. It is commonly used, however, for wildlife or field anaesthesia (Cohen and Brae, 1978).

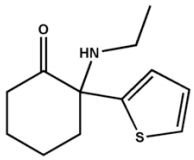


Fig. 8: Tiletamine (HCl)

2.3 Ketamine

2.3.1 History

Ketamine hydrochloride was first synthesised in the 1962 in the Parke Davis Lab (today a part of the pharmaceutical company Pfizer) by C. Stevens and was originally named CI-581 (CI state for Clinical Investigation). Stevens was in search for a safer alternative to PCP. Ketamine was first patented in 1963 under a Belgian and years later (1966) under US patent as an anaesthetic for humans and animals. In 1970, ketamine was approved by the Food and Drugs Administration and ketamine became commercially available as Ketalar ®.

Ketamine was originally used as an anaesthetic in the Vietnam War to anaesthetise American soldiers (Mellor, 2005). This experience suggested that ketamine could be useful in civilian contexts to provide anaesthesia or immobilisation for outdoor life-saving

emergency surgeries or in mass casualties where rapid procedures were required (Bonanno, 2002).

Ketamine is a very versatile and inexpensive drug. In regions where access and funding (as in the developing world) for a wider range of drugs is problematic, its broad range of clinical applications is ideal. Furthermore, its favourable safety profile and ease of storage makes it ideal for use in areas where refrigerators, complex monitoring, electricity and oxygen may all be scarce or unreliable (Craven, 2007).

The first veterinary use is already described in 1972 for chemical restrain of nonhuman primates (Bree *et al.*, 1972).

2.3.2 Chemistry: Isomerism of ketamine

Ketamine is a keto-amine, so named as it has a ketone C=O group bonded to carbons either side, as well as an amine group. As such it resembles NMDA (Fig. 9 and 10)

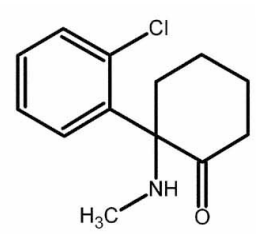


Fig. 9: Ketamine

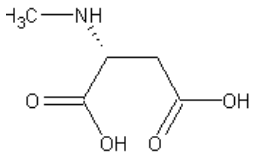
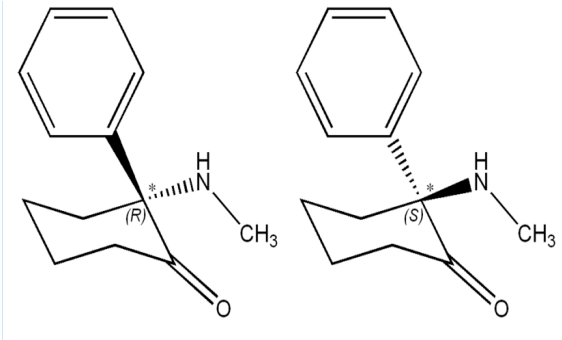


Fig. 10: NMDA

Ketamine contains a chiral centre at the C-2 carbon of the cyclohexanone ring, so that two enantiomers exist S (+) ketamine and R (-) ketamine (Fig. 11).



R (-) ketamine S (+) ketamine

Fig. 11: Two isomers of ketamine

2.3.3 Pharmacokinetics

The use of ketamine, administrated by infusion or injection, in the proximity to the spinal cords, i.e. Intrathecally, into the CSF or epidural into the fatty tissue surrounding the dura (also known as neuroaxial drug administration techniques) is in various stages of investigation (Schug *et al.*, 2006).

Thanks to its good lipid solubility, ketamine crosses the brain-blood barrier quickly and in short time (less than one minute) its action in CNS starts (Cohen et al., 1973). Racemic ketamine has relatively short distribution and elimination half-lives: the alfa-elimination phase last only a few minutes, and the beta-elimination half-life is 2-3 hours (Kohrs and Durieux, 1998).

The compound is metabolised by hepatic cytochrome p450 system, where it is bio transformed by N-demethylation to norketamine (metabolite 1), which retains the pharmacological activity and then undergoes oxidation of the cyclohexanone ring to form dehydronorketamine (metabolite 2) (Bolze and Boulieu, 1998; Seay *et al.*, 1993*)*. Its primary metabolite norketamine is only 1/3 to 1/5 as potent as the original compound but might be involved in the prolonged analgesic actions of ketamine. The metabolites are excreted by the kidney *(*Roncada *et al.*, 2003; Kohrs and Durieux, 1998).

Humans. A variety of pharmacokinetic studies on ketamine using 2 or 3 compartments models are available (Hijazi *et al.*, 2003).

Animals. The comparison of the pharmacokinetics of ketamine HCl in cats, dosed at 25 mg/kg, after IV and IM, and rectal administration, showed rapid absorption with all three ways of administration (Hanna *et al.*, 1988). The rectal administration of Ketamine HCl showed a minimal influence of the first–pass effect and a statically similar elimination rate (beta) in rats (White and Holmes, 1976), dogs (Schwieger *et al.*, 1991), and horses (Muir and Sams, 1992; Waterman *et al.*, 1987).

Ketamine can be applied PO (Grant *et al.*, 1984), IM, SQ, IV, intra nasal (Axiak *et al.*, 2007), rectally (Kruger, 1998 and Hanna *et al.*, 1988), but tissue irritation may occur after IM injection site (Booth, 1988).

2.3.4 Pharmacodynamics

2.3.4.1 Action of ketamine

In the brain, ketamine acts on receptors located in the thalami-neocortical projection system, where it depresses neuronal function of the neocorticothalamic axis and the

central nucleus of the thalamus, while it stimulates parts of the limbic system, including hippocampus. The result is dissociation between these brain areas (Miyasaka and Domino, 1968).

Hallucinatory behaviour, which may progress to delirium, may occur during emergence from anaesthesia. Ketamine-induced depression of the inferior colliculus and medial geniculate nucleus leading to misperception of auditory and visual stimuli may be responsible for this reaction (White, 1982).

In dogs ketamine, when used alone, induces extreme muscle tone, exuberant spontaneous movement, violent recovery, and occasional convulsions. In "high-strung" small breed dogs, small ketamine doses produce insufficient anaesthesia and have greater tendency to cause seizures. Excessive salivation may occur during ketamine anaesthesia, but this can be controlled with atropine (Navarro and Friedman, 1975).

2.3.4.2 Receptors of ketamine

At a molecular level, ketamine binds to various receptors:

- NMDA-Glutamate receptors
- Nicotine cholinergic receptors
- Monoaminoergic receptors
- Opioids receptors
- Muscarin cholinergic receptors
- Voltage-dependent ion channels such as Na and L-Type Ca Ions channel

2.3.4.2.1 NMDA glutamate receptors

N-Methyl-D-aspartate (NMDA) receptors are members of the glutamate receptors channel super family, which mediate most of the fast excitatory synaptic transmissions in the central nervous system.

The NMDA receptor is highly permeable to calcium and sodium ions and plays a key role in the plasticity of synapses, which is believed to underlay memory and learning, as well as the development of the nervous system.

This receptor opens in response to binding of the neurotransmitter glutamate. Besides the glutamate (NMDA) binding site, there are also multiple binding sites on the NMDA receptor for glycine and polyamines. NMDA receptors possess 4 trans membrane helical domains (Fig. 12) and can be blocked by magnesium ions, inhibited by zinc ions, and modulated by arachidonic acid.

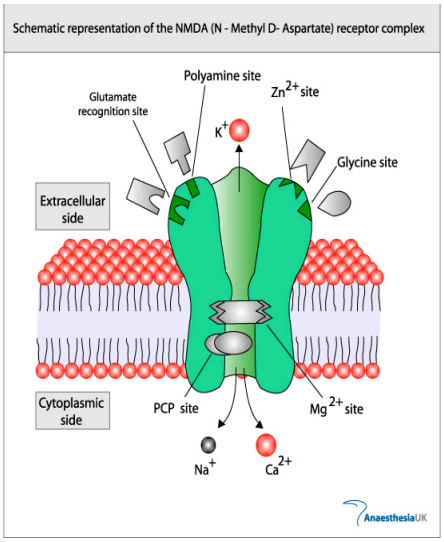


Fig. 12: Scheme of a NMDA Receptor which mediates most of the fast excitatory synaptic transmissions in the central nervous system

At the spinal cord level, NMDA receptor activation triggers a cascade of events leading to sensitisation of dorsal horn wide dynamic range neurons. There is a significant increase in intracellular calcium and activation of protein kinases and phosphorylating enzymes. NMDA receptor stimulation will also increase the production of spinal phospholipase and induce the production of nitric oxide synthetase. The prostaglandins and nitric oxide, which are subsequently produced and released into the extra cellular milieu, can facilitate further release of excitatory amino acids and neuropeptides from primary afferent pain fibres.

Ketamine inhibits the NMDA receptor by binding in a non-competitive manner to its PCP site. The blockade is time, concentration, and stimulation frequency dependent (use dependent) (MacDonald *et al.*, 1987).

2.3.4.2.2 Opioid receptors

It is generally believed that NMDA receptor antagonism accounts for most of ketamine anaesthetic and analgesic effects, where as ketamine agonistic effects at opioid receptors within the central nervous system seem to play a minor role in its anaesthetic or analgesic effect (Hustveit *et al.*, 1995).

The psychomimetic side effect of ketamine may be explained by the interaction with κ-opioid receptors, because κ-agonist produces similar effect. Its low affinity for these receptors compare to NMDA receptors suggest that the interaction is not of major clinical importance (Kohrs and Durieux, 1998).

Few studies have assessed the involvement and contribution of the Π-opioid receptor in generating ketamine respiratory effects (Latasch and Freye, 1993). Opioid receptors and their endogenous ligands are found in large concentrations in areas of the CNS

involved in the control of breathing. Administration of ketamine results in respiratory depression, probably because of its interaction with П–opioid receptors (Sarton *et al.*, 2001).

2.3.4.2.3 Cholinergic and adrenergic signalling: nicotine- and muscarin-cholinergic receptors

Both receptors (nicotine- and muscarin- cholinergic receptors) are affected by ketamine (Kress, 1994).

The effect of ketamine on human nicotinic cholinergic receptors is inhibition depending on type of subunit receptors (Yamakura *et al.*, 2000).

The postsynaptic inhibitory effect of ketamine on nicotinic acetylcholine receptors in skeletal muscle is not necessarily noticeable clinically, as ketamine increases muscle tone by central mechanisms. Muscarinic receptors are also inhibited (Durieux, 1995). S (+) ketamine shows a twofold greater affinity with the muscarinic receptor than does R (-) ketamine (Hustveit *et al.*, 1995). However, overall affinity for the muscarinic receptor is ten to twenty folds less than NMDA receptor binding (Aronstam *et al.*, 1982). Emergence side effects may be partly related to inhibition of cholinergic transmission (Durieux, 1995).

Ketamine profoundly inhibits muscarinic signalling. This effect might explain some of the anti cholinergic clinical effects of ketamine, both: central (effects on memory and consciousness) and peripheral (prominent sympathetic tone, bronchodilatation, mydriasis) as stated by Durieux (1995).

2.3.4.2.4 GABA receptors

GABA is the most common inhibitory neurotransmitter in the CNS, and GABA signalling neurones account for approximately 30% of all synaptic connections in the CNS (Kohrs and Durieux, 1998).

Although the effect of ketamine as GABA signalling seems established (Lin *et al.*, 1992), the concentrations used in the study were higher than those used normally in the clinic. Based on current knowledge, this interaction seems to be of minor importance for clinical practice (Kress, 1994).

2.3.4.3 Effect on different organ system

2.3.4.3.1 Effect on the central nervous system

Ketamine produces a dose related unconsciousness and analgesia (Cohen *et al.*, 1973).

Cerebral blood flow (CRF) and Intracranial pressure (ICP). Cerebral blood flow (CBF): ketamine racemic form and S (+) form, produced similar EEG changes (Thiel *et al.*, 1992).

Hallucinations during recovery. The ketamine induced depression of the inferior colliculus and medial geniculate nucleus leading to misperception of auditory and visual stimuli may be responsible for this reaction. In cats emergence reactions are characterised by ataxia, increased motor activity, hyperreflexia, sensitivity to touch, avoidance behaviour of an invisible object ('watching butterflies') and sometimes violent recovery (Wright, 1982). The combination of ketamine with alpha-2-agonists, acepromazine or a benzodiazepine derivate, decrease the incidence of adverse emergence reaction (Lin *et al.*, 1992). At low doses, stimulant effects predominate and the effect on environmental conditions is significant; with higher doses, psychedelic effects predominate and the effect of the environment diminishes (Wolff and Winstock, 2006).

Ketamine is not approved in the Unites States for patients less than 16 years of age. Ketamine induces neuronal injury and/or death in the brains of juvenile rodents (Ikonomidou *et al.*, 1999 and 2001; Jevtovic-Todorovic *et al.*, 2000). In a review study of environmental agents (like ethanol, PCP, ketamine, benzodiazepine, halothane, isoflurane, and propofol) that have the potential to trigger apoptosis of neurones in developing brain, the authors invite to further research. During the synaptogenesis period (from the last trimester of pregnancy and several years after birth in humans) the transient interference in the activity of GABA and NMDA receptors causes neurodegenerative cell death (Wang and Slikker, 2008 and Olney *et al.*, 2000). Scallet *et al.* (2004) confirmed that ketamine has been shown to induce neuronal death in rats during early development. The effects of ketamine are dose dependent; data suggest that limiting exposure limit the potential for neurodegeneration. In animal models the doses and durations of ketamine exposure that resulted in neurodegeneration were slightly larger than those used in the clinical setting, but there are insufficient data to either support or refute the clinical applicability (Mellon *et al.*, 2007). Most of the recent preview studies, have the goal to warn clinicians of use of ketamine because of its neurotoxicity (Lois and De Kock, 2008; Vutskits *et al.*, 2007;).

Ketamine has been shown to have neurotoxic properties, when administered neuraxially, at millimolar concentrations ketamine induces apoptosis via the mitochondrial pathway, independent of death receptor signalling. At higher

concentrations necrosis is the predominant mechanism. Less toxicity of S (+) ketamine was observed in neuroblastoma cells, but this difference was minor and therefore unlikely to be mediated via the NMDA receptor (Braun *et al.*, 2010).

Ketamine use in children's anaesthesia indicates interesting properties such as the prevention of surgery and opiate induced hyperalgesia and the anti-proinfiammatory characteristic (by promoting the self limitation of the inflammatory response that follows surgery). This positive effects of ketamine are contrasted by other more potentially deleterious effects proven by experiments on rats treated with ketamine at anaesthetic doses during the critical period for the central nervous system development, where long-term behavioural deficits were noted (Lois and De Kock, 2008).

2.3.4.3.2 Effects on cardiovascular system

Indirect cardiovascular stimulation. Following ketamine heart rate and arterial blood pressure increase as a result of direct CNS stimulation. Plasma concentration of epinephrine and norepinephrine increase within 2 min after IV administration of ketamine. As a consequence heart rate, cardiac output and arterial blood pressure increase following administration (Brake *et al.*, 1973).

In a study reporting cardiovascular response after intracisternal injection of ketamine or saline vehicle, a dose dependent increase in blood pressure and heart rate was observed (0.5 or 1.0 mg). In contrast, higher dose of ketamine (4 mg) produced a fall in blood pressure and heart rate (Seth *et al.*, 1990).

Inotropic action on myocardium. The inotropic effect is controversial. Some studies (in vivo and in vitro) support the thesis of positive inotropic effect (Cook *et al.*, 1991; Riou *et al.*, 1990; Riou *et al.*, 1989; Tweed *et al.*, 1972). Others, however, support the negative inotropic effect (Kongsayreepong *et al.*, 1993; Rusy *et al.*, 1990; Goldberg *et al.*, 1970; Dowdy and Kaya, 1968). In anaesthetic doses, ketamine may inhibit catecholamine beta-adrenergic activity, negating a direct depressant effect upon the heart (Reyes Toso *et al.*, 1992).

Ketamine is used in patients suffering from hemodynamic shock, active asthmatic disease, and anaesthesia in children and intensive care settings (Kohrs and Durieux, 1998).

2.3.4.3.3 Effects on respiratory system

Respiration pattern. Ketamine provokes only mild respiratory depression (Werner *et al.*, 1997). Ketamine is one of the most reliable anaesthetic agents for anaesthesia induction and maintenance, since at clinically useful dose rates, it does not impair airway maintenance and spontaneous respiration (Paix *et al.*, 2005). The transient apnoea induced by ketamine appears to be dose dependent. At higher doses, respiration is characterised by an apneustic, shallow, and irregular pattern (Sears,

1971). Severe depression or arrest with over dosage has been described in humans and cats (Bree *et al.*, 1972; Child *et al.*, 1972; Kopman, 1972).

Secretion from salivary glands and respiratory tract mucus. Ketamine can cause increased salivation and secretion of respiratory track mucus, which can easily be controlled by administration of an anti-cholinergic (Wright, 1982).

Reflexes. Laryngeal and pharyngeal reflexes are well maintained during ketamine anaesthesia. Nevertheless, most species can be intubated when anaesthetised with ketamine in combination with other anaesthetic agents or sedatives (Wright, 1982).

Pulmonary capillary effect. An analysis of ketamine responses in the pulmonary vascular bed of the cat showed that ketamine had a significant vasodilator activity in the pulmonary vascular bed (Kaye *et al.*, 2000).

2.3.4.3.4 Inhibition of inflammatory response

There are promising effects of ketamine on the inflammatory response in vitro and in vivo. Intravenous anaesthetics generally depress the endotoxin-induced pro-inflammatory cytokine activity and its consequent nitric oxide generation, free radicals production and neutrophil activity. Different intravenous anaesthetics, such as propofol, ketamine, benzodiazepine and barbiturates, which produce different levels of inhibition of inflammatory effects, may be of great relevance to the practice of intensive care, and intravenous anaesthetics may play significant roles in this regard (Lois and De Kock, 2008; Tsao *et al.*, 2005).

2.3.4.3.5 Analgesia

Many mechanisms are at the origin of ketamine analgesic property:

- Blockade of the spinoreticular tract (Sparks *et al.*, 1973).
- Depression of nuclei of the medial medullary reticular formation in the cat (Ohtani *et al.*, 1979).
- Suppression of lamina of the spinal cord (Kitahata *et al.*, 1973);
- Interaction with CNS and spinal cord opiate receptors (Hanaoka et al., 1990; Smith et al., 1990; Finck and Ngai, 1982).
- NMDA receptors antagonism.

The degree of analgesia appears to be greater for somatic pain than for visceral pain (Haskins *et al.*, 1975 and Sawyer *et al.*, 1991). In the cat analgesic effect of ketamine (at a dose of 4 mg/kg IV) in visceral pain is similar to the effect covered by butorphanol (at a dose of 0.1 mg/kg IV), (Sawyer *et al.*, 1990). Ketamine has analgesic characteristics even at sub anaesthetic dosages (Clements and Nimmo, 1981). Recently, the use of ketamine in form of chronic administration for analgesia was investigated (Ben-Ari *et al.*, 2007). The primary role of ketamine in such low doses

consists in 'anti-hyperalgesia', 'anti-allodynia' or 'tolerance-protection'. Therefore, ketamine plays a role in the treatment of opioid resistant or 'pathological' pain (central sensitisation with hyperalgesia or allodynia, opioid induced hyperalgesia, neuropathic pain) rather than as an 'analgesic' in its own right. Low dose ketamine also has 'preventive analgesia' properties (Visser and Schug, 2006) as it prevents central sensitisation. Furthermore, in higher doses it provides effective and safe sedation and analgesia for painful procedures (Visser and Schug, 2006).

Acute pain management. Pyati and Gan (2007) reviewed the evidence on the opioid-sparing effect of ketamine and other analgesic drugs in the peri-operative pain management. According to the authors, most available data support the addition of these molecules to routine analgesic techniques to reduce the need for opioids and improve the quality of analgesia by their synergistic effect.

Chronic pain management. NMDA agonists such as ketamine are well known to attenuate central sensitisation and palliate neuropathic pain (Okon, 2007).

Ketamine has been successfully used in patients with advanced cancer that resisted to ordinary pain therapies. Ketamine was infused at slow rate from over 6 hours to 48 days and only few patients showed disorientation and drowsiness during infusion, but no cardiovascular or respiratory complication (Kanamaru *et al.*, 1990). In a recent review, ketamine counts to the various analgesics in use to control cancer pain (Bell, 2009; Newsome *et al.*, 2008).

2.3.5 Club drugs: Recreational use of ketamine

Club drugs are substances commonly used at nightclubs, music festivals, raves, and dance parties to enhance social intimacy and sensory stimulation. The most widely used club drugs are 3,4-methylenedioxymethamphetamine (MDMA), also known as ecstasy; gamma-hydroxybutyrate (GHB); flunitrazepam (Rohypnol ®); and ketamine (Ketalar ®). These drugs are popular because of their low cost and convenient distribution as small pills, powders, or liquids. Club drugs usually are taken orally and may be taken in combination with alcohol, or with other drugs (Gahlinger, 2004).

The incidence of recreational ketamine use increased through the end of the 20th century, especially in the context of raves and other parties. Production for recreational use has been traced to 1967, when it was referred to as "mean green" and "rockmesc". Recreational names for ketamine include "K", "Ket", "Special K", "Vitamin K" and "King Kev".

At low doses, stimulant effects predominate and the effect of environmental conditions is significant; with higher doses, psychedelic effects predominate and the effect of the

environment diminishes. The potential of ketamine as a novel clinical and research tools matched by its abuse potential outside medical settings (Wolff and Winstock, 2006).

2.4 S (+) ketamine

2.4.1 History

For many years, racemic ketamine has been used widely in human and veterinary medicine, but collateral effects have been described for the racemate, like cardiovascular stimulation and hallucinations (White, 1982).

2.4.2 Use in animals and humans

S (+) ketamine is used for premedication, sedation, induction and maintenance of general anaesthesia, which is than termed "dissociative anaesthesia". S (+) ketamine is an ideal anaesthetic agent for trauma victims, patients with hypovolemic and septic shock and patients with pulmonary diseases. Even sub anaesthetic doses of this drug have analgesic effects, so ketamine is also recommended for post-operative analgesia and sedation (Sinner and Graf, 2008).

S (+) ketamine at half-dose of racemic ketamine is as potent as racemic ketamine in sub anaesthetics doses with powerful analgesics properties. S (+) ketamine exerts less adverse effects on measurable cerebral function and induces a significantly smaller increase in heart rate. Since states of impaired consciousness and disorientation are especially disturbing under emergency conditions, the use of S (+) ketamine as a potent analgesic for therapeutic use in emergency and disaster medicine deserves further investigation (Pfenninger *et al.*, 1994).

In humans S (+) ketamine widens the anaesthesiologist's possibilities of premedication considerably. Induction of anaesthesia with stabilised spontaneous ventilation becomes in fact possible in children with difficult condition for venous puncturing, in very anxious children, and in those who are not able to accept the necessity of a treatment (Kruger, 1998).

S (+) ketamine binds approximately two to fourfold stronger to opioid receptors than does R (-) ketamine, still the affinity of ketamine for these receptors is 10 (μ) to 20 (k) times less than for NMDA channel. This suggests that this interaction is not of clinical importance (Hustveit *et al.*, 1995).

Veterinary medicine. The 18th of Mai 2006 Swissmedic gave the authorisation for the use and trade of S (+) ketamine in cats. The pharmaceutical company Dr. E. Graeub AG

became the first company to register the S (+) ketamine is under the trade name Keta S ®, ad us vet.

Cats. A lower dose of S (+) ketamine was necessary compared to racemic ketamine for elective ovarioectomy (Eichenberger *et al.*, 2005).

S (+) ketamine has been successfully used in ASA III cat patients offering significantly faster recovery period than the racemic mixture. Post-operative analgesia and emergence quality were also judged significantly better (Baumgartner *et al.*, 2002).

In male cats undergoing routine neutering surgery, anaesthesia with S (+) ketamine, at a 60% the dose of racemic ketamine, provide faster recovery, increased post operative respiratory rates, and adequate post operative analgesia (Larenza *et al.*, 2008).

Horses. In study comparing the effect of plasma concentrations obtained by a low dose constant rate infusion (CRI) of racemic ketamine or S (+) ketamine on the nociceptive withdrawal reflex (NWR) in standing ponies, the NWR was significantly depressed in the racemic ketamine group at plasma concentrations between 20 and 25 ng/mL of each enantiomer. No significant NWR depression in the S (+) ketamine group could be observed. The antinociceptive activity in standing ponies, demonstrated as a depression of the NWR, could only be detected after treatment with racemic ketamine. S (+) ketamine may have lacked this effect as a result of lower plasma concentrations, a more rapid metabolism or a lower potency of S (+) ketamine in horses (Peterbauer *et al.*, 2008).

The plasma concentrations of ketamine and norketamine enantiomers were determined by capillary electrophoresis. The residence times were lower for S (+) ketamine after S (+) ketamine administration compared to racemic ketamine. The time to standing position (TSP) during recovery was significantly shorter after S (+) ketamine administration compared to racemic ketamine (Larenza *et al.*, 2008) at equipotent doses.

In an evaluation of the pharmacokinetics of ketamine and norketamine enantiomers, Larenza *et al.* (2007), found that the plasma concentrations of S (+) ketamine decreased and biodegradation products increased more rapidly after S (+) ketamine CRI as compared with racemic ketamine CRI, indicating that S-enantiomer was eliminated faster when infused alone instead of as part of a racemic mixture. Furthermore, the recovery from anaesthesia was better with S (+) ketamine infusions as compared with racemic ketamine (Larenza *et al.*, 2009 and 2008). Another study in Shetland Ponies confirmed these results showing a faster recovery after administration of S (+) ketamine as compared with its racemic mixture (Larenza *et al.*, 2009 and 2007).

Theurillat et al., (2005), describe a method for the simultaneous determination of the enantiomers of ketamine and norketamine in equine plasma. The ketamine N-demethylation was demonstrated to be enantioselective and the concentrations of the

two ketamines enantiomers in plasma were equal, whereas S (+) norketamine was found in a larger amount than R (-) norketamine.

Pig. A bolus injection of S (+) ketamine was associated with less cerebral and systemic hemodynamic depression than racemic or R (-) ketamine in equipotent doses an experimental model. These findings indicate possible advantages of S-ketamine over racemic ketamine (Schmidt *et al.*, 2005).

Dogs. The application in dogs of a smaller dose of S (+) ketamine will produce a shorter time to sternal recumbency and a shorter time of recovery from anaesthesia when compared to racemic ketamine (Duque *et al.*, 2008).

In dogs the use of S (+) ketamine anaesthesia resulted in the shorter duration of unconsciousness, shortest time to sternal recumbency (TSR) and more quiet recovery from anaesthesia when compared to racemic ketamine (Muir and Hubbell, 1988). The application of a smaller dose of S (+) ketamine, in comparison with racemic ketamine, produced a faster recovery from anaesthesia with a minor incidence of psychomimetic side effects, at similar cardiovascular effects during anaesthesia (Oleskovicz *et al.*, 2009; de Almeida *et al.*, 2005; Duque *et al.*, 2004). The R (-) enantiomer is a more potent bronchodilator than the S (+) ketamine during ACh-mediated ASM contraction. This effect appears to be caused by differential actions on receptor-operated calcium Channels (Pabelick *et al.*, 1997).

The potency ratio between racemic ketamine and S (+) ketamine in dogs is smaller than reported in other species. A dose reduction of 50% of S (+) ketamine in comparison to racemic ketamine, as usually performed in humans, does not result in equipotent anaesthetic effects in dogs (Duque *et al.*, 2004).

2.4.3 Effects on different organ system

CNS effects. Racemic ketamine and S (+) ketamine produce similar EEG changes in the first phase of after injection. However, 120 min after injection the EEG shows more activity following racemic ketamine as compared with S (+) ketamine. Although, a statistically significant difference could not be confirmed, a better recovery of cortical function after S (+) ketamine administration is probable (Thiel *et al.*, 1992).

Cardiovascular effects. Ketamine and its isomers were found to act as cardiac depressants by affecting calcium homeostasis in isolated ferret and frog ventricular myocardium (Kongsayreepong *et al.*, 1993). However, as Graf *et al.* (1995) have reported using the isolated perfused heart model, S (+) ketamine is less depressant compared to racemic and isomeric R (-) ketamine. A later study of Kunst et al. (1999) on human myocardium concluded that S (+) ketamine at lower doses (73 microM) increased isometric force, isotonic shortening, contractility, and relaxation. At higher doses (730 microM) a direct inotropic action was observed after perfusion with ketamine

and isomers, which was accompanied by decreased intracellular Ca^{2+} transient (Kunst *et al.*, 1999).

Pain. S (+) ketamine is approximately twice as potent as the racemic mixture in inhibiting central summation of pain (Arendt-Nielsen *et al.*, 1996). In this way, the S (+) isomer offers advantages in the clinical use by using equipotent doses causing less collateral effects than the racemic ketamine. S (+) ketamine has an opioid-sparing effect after cardiac surgery. The analgesic property of S (+) ketamine was also supported by the finding that the time to the first request for an analgesic after surgery was longer in the ketamine group (Lahtinen *et al.*, 2004). S (+) ketamine at half dose of racemic ketamine is as potent as racemic ketamine in sub anaesthetic doses with powerful analgesic properties (Pfenninger *et al.*, 1994).

Recovery. White *et al.* (1985) showed that patients felt more comfortable after S (+) ketamine anaesthesia compared with the racemic mixture, mainly because of decreased agitation, disorientation, and anxiety. The effect on vigilance was less impaired by S (+) ketamine, psychological assessment showed a prompter recovery of visual attentiveness and sensimotor performance in the S (+) ketamine group, and subjective mood was judged by volunteers to be significantly better after S (+) ketamine (Doenicke *et al.*, 1992). The additional administration of a benzodiazepine provided both drugs with a significantly higher rate of acceptance, but also an increased duration of recovery (Kohrs and Durieux, 1998). Taken together, these data suggest that S (+) ketamine allows for the reduction of the anaesthetic doses, with a resultant faster recovery and (possibly) some diminution in side effects (Kohrs and Durieux, 1998).

2.5 Anaesthetic consideration for elective orchiectomy in dogs

General anaesthesia is mandatory for elective surgeries involving the reproductive tract under the application of local anaesthesia. Numerous general anaesthesia protocols may be used for elective surgery in healthy animals (ASA I and II).

2.5.1 Dissociative anaesthesia using diazepam or xylazine and ketamine

- Diazepam (Vallium ®) 0.05-0.25 mg/kg IV has sedative, anti-convulsive, anti-arrhythmic and muscle relaxation properties. At high doses it produces hypnosis ad ataxia.
- Xylazine (Rompun ®) 0.5-2 mg/kg IM produces sleep-like symptoms, good analgesia and good muscle relaxation.
- Ketamine (Ketanest ®; Ketalar ®) 10-30 mg/kg IM gives analgesia and anaesthesia

During 1993, 66 small animal practices of Ontario, participated in a prospective study to evaluate the incidence and details of anaesthetic-related morbidity and mortality (Dyson *et al.*, 1998). Considering a total of 8,087 dogs, the incidences of complications were 2.1% and death occurred in 0.11% of cases. Significant complications in dogs were associated with xylazine, heart rate monitoring, ASA 3 or more patients. Cardiac arrest in dogs was associated with xylazine use, while the use of ketamine was associated with high safety (Dyson *et al.*, 1998).

2.5.2 Dissociative anaesthesia using tiletamine and zolazepam

Powder mixture contains 250 mg of tiletamine and 250 mg of zolazepam (Telazol ®) in 5 ml solution. Telazol is expensive and once mixed it can't be stored for a long time.

The dose can vary from 7 to 25 mg/kg BM by IM administration and from 5 to 10 mg/kg by IV administration respectively, depending on anaesthesia indication and purpose (immobilisation to small surgery procedure). Those combinations fail to induce a surgical level of anaesthesia (Hellebrekers *et al.*, 1998).

2.5.3 Dissociative anaesthesia using medetomidine, butorphanol and ketamine

- In Switzerland, since 2007, the pharmaceutical company Dr. E. Graeub Veterinary products, provides the veterinary clinician with a practical table (Table 1) that help the routine use of the combination of butorphanol (Morphasol ®), Medetomidine (Dorbene®), and racemic ketamine (Ketasol®).

- Table 1: Protocol for combination of Morphasol ® - Dorbene® - Ketasol®

	Sedation					Anaesthesia		
Dog	Morphasol-4	Dorbene				Ketasol-100		
	4 mg/ml Butorphanol	1 mg/ml Medetomidine				100 mg/ml Ketamine		
		IM		IV		Induction dose	Maintenance dose	
kg BM	0,2 mg/kg BM	mild sedation	deep sedation	mild sedation	deep sedation	5 mg/kg BM	3 mg/kg BM	
1	0,05	0,04	0,10	0,03	0,08	0,05	0,03	15 minutes later
2	0,10	0,06	0,16	0,04	0,12	0,10	0,06	
3	0,15	0,08	0,21	0,06	0,16	0,15	0,09	
4	0,20	0,09	0,25	0,07	0,19	0,20	0,12	
5	0,25	0,11	0,30	0,08	0,22	0,25	0,15	
6	0,30	0,13	0,33	0,09	0,25	0,30	0,18	
7	0, 35	0,14	0,37	0,10	0,28	0,35	0,21	
8	0,40	0,15	0,40	0,11	0,30	0,40	0,24	
9	0,45	0,16	0,44	0,12	0,33	0,45	0,27	
10	0,50	0,18	0,47	0,13	0,35	0,50	0,30	
12	0,60	0,22	0,53	0,15	0,40	0,60	0,36	
14	0,70	0,24	0,59	0,16	0,44	0,70	0,42	
16	0,80	0,26	0,64	0,18	0,48	0,80	0,48	
18	0,90	0,28	0,69	0,19	0,52	0,90	0,54	
20	1,00	0,30	0,74	0,21	0,56	1,00	0,60	
22	1,10	0,32	0,80	0,22	0,60	1,10	0,66	
24	1,20	0,34	0,86	0,24	0,64	1,20	0,72	
26	1,30	0,36	0,90	0,25	0,67	1,30	0,78	
28	1,40	0,37	0,94	0,26	0,70	1,40	0,84	
30	1,50	0,37	0,98	0,27	0,73	1,50	0,90	
32	1,05	0,40	1,03	0,28	0,77	1,60	0,96	
34	1,10	0,42	1,08	0,30	0,80	1,70	1,02	
36	1,15	0,42	1,12	0,31	0,83	1,80	1,08	

From: "Dosierungstabelle Anästhesie mit Morphasol-4", Graeub , Veterinary Products, Switzerland, Doc 151.53/06.2007

- Medetomidine (Domitor ®, Dorbene ®) 450 μg/m2 BSA (body surface area), used intramuscularly (IM)

- Butorphanol (Morphasol ®, Dolorex ®, Alvegesic 1% forte ®) dose 0.2 mg/kg BW, used intramuscularly (IM)

- Racemic ketamine (Ketasol®, Narketan ® 10) Racemic Ketamine 4 mg/kg BW intravenously (IV)

A study in dogs (Ko *et al.* 2000), suggested that the combination of medetomidine with butorphanol or ketamine resulted in more reliable and uniform sedation in dogs as compared with medetomidine alone.

In a study of Girard *et al. (*2010) the sedative effects of low-dose medetomidine, butorphanol, and their combination were tested in dogs. The combination of low-dose medetomidine (1 μg/kg IV) and butorphanol (0.1 mg/kg IV) produced a stronger

sedation than medetomidine or butorphanol alone. Heart rate was significantly decreased in both medetomidine groups (Girard *et al.*, 2010).

2.5.4 Anaesthesia using propofol

Propofol in Switzerland is sold under the trade name Disoprivan®, and it is registered for the human medicine use.

Propofol can be used in dogs in the dosage of 4-8 mg/kg IV, this dose must be reduced to 4-5 mg/kg after acepromazine, and to 1-2 mg/kg after medetomidine premedication, respectively. Propofol can be use for anaesthesia induction and for anaesthesia maintenance (Glowaski *et al*., 1999; Pascoe, 1992).

In a study of Hellebrekers *et al.* (1998), the clinical efficacy and safety of propofol and of racemic ketamine was compared. A higher rate of adverse side effects related to the use of racemic ketamine was reported.

2.6 Potential economical / research impact

In 1992, the Food and Drug administration stated that separation of stereoisomers did not receive appropriate attention in commercial drug development and that, despite technical difficulties and high cost, this issue could open new horizons in therapeutics (Kohrs and Durieux, 1998).

About 56% of the drugs currently in use are chiral compounds, and 88% of these chiral synthetic drugs are used therapeutically as racemate. If the qualitative and quantitative pharmacokinetic and pharmacodynamic effects are similar, the enantiomers do not need to be separated. However, if different enzymes handle the metabolism of the different stereoisomers, and if their pharmacodynamic effects have differences either in strength or in quality, enantiospecific analysis is urgently needed. Therefore, there is a great need for studies focussing on these differences to improve therapeutic applications of chiral drugs (Rentsch, 2002). This may results in higher therapeutic and cost effectiveness.

3. Methods

3.1 Study design

3.1.1 Overall design

The experimental study was designed as a randomised blinded prospective clinical trial according the VICH guidelines on Good Clinical Practice (GCP) and approved by the Cantonal Veterinary Services of Canton Vaud.

Two different types of ketamine (racemic ketamine: Ketasol-100 ad us. vet, and S (+) ketamine isomer: Keta-S ad us. vet were tested. Their intra-operative hemodynamic effects and ability to provide an adequate anaesthesia induction, maintenance and recovery were assessed in male dogs undergoing elective neutering.

3.1.2 Treatment for control group

As a treatment control, a ketamine racemate (Ketasol-100 ad us. vet) group was provided. However, the protocol for treatment did not differ for the control group.

3.1.3 Randomisation method

A total of 40 male dogs (client owned or belonging to the local animal shelter) classified as ASA I to II risk patients according to the American Society of Anaesthesiologist (ASA) were included in the trial. The randomisation was carried out by a supporting person (a veterinarian). The supporting person made use of the envelope system provided by the sponsor. This person also prepared the syringes of racemic ketamine or S (+) ketamine.

3.1.4 Experimental units

Two different groups (A and B) were formed, containing 20 animals each. One group was given S (+) ketamine and the other was given racemic ketamine.

3.1.5 Blinding

Blinding was ensured by the supporting person, who was responsible for keeping the blinding records and for preparing the test drugs. The supporting person also handled the syringes to the investigator, ready to be used. The investigator remained unaware of the treatment identity during the entire animal phase.

3.2 Inclusion/exclusion criteria and removal after removal

3.2.1 Pre-admission

Careful physical examination was performed prior to the anaesthesia induction (See Annex A: Pre-admission examination). Dogs were classified according to the American Society of Anaesthesiologists' (ASA) physical status classification in ASA risk groups I, II, III, IV and V.

3.2.1.1 Inclusion criteria

- ASA I-II
- Non-sterilised male dogs

3.2.1.2 Exclusion criteria

- non-cooperative dogs
- patients with pre-anaesthetic pathological disorders
- cryptorchids

Notice: ASA I or II means the dogs are suitable to receive general anaesthesia

3.2.2 Removal after inclusion

- Inability to induce anaesthesia with the pre-established drugs and doses
- Inability to perform the pre-established surgery (surgical complications)

4. Procedures

4.1 Key study dates

The timeframe per case was approximately 3 hours, including clinical examination, anaesthesia pre-medication, animal preparation, anaesthesia induction, orchiectomy, monitoring and post-operative recovery and observation. After orchiectomy, all animals were observed and filmed during one hour.

4.2 Animal selection and identification

A total of 40 dogs entered the study. Animals were recruited from private clients of the veterinary practice, as well as from the local animal shelter (Société Vaudoise de la Protection des Animaux -SVPA-). The selection was based on a clinical pre-admission examination: anamnesis, physical exam (including: weight, hydratation status, temperature), cardiovascular and respiratory system (respiratory frequency, heart rate, mucous membrane colour, capillary refill time, pulse character, thoracic auscultation) and on specific inclusion/exclusion: risk classification of the American Society of Anaesthesiologists criteria (ASA, ASA I-II: low risk; ASA III: moderate risk, ASA IV-V: high risk).

Each animal was identified (given name, owner’s surname, breed, age, sex); each dog received a serial study number, which was marked on its dossier as well as on the tape video document.

At the end of each completed trial the patients’ dossier was stapled together to form a single file and stored safely by the investigator.

4.3 Animal management and housing

Private owners or SVPA provided all animals after having been informed on trial procedures and general anaesthesia risks and having signed the respective form (owner's consent, "Consentement du propriétaire"). The animals returned home on the same evening. The animals were kept in conventional kennels until anaesthesia induction and after completion of the visual examination (1 hour after surgery). A quiet environment at room temperature was granted during all clinical trials.

4.4 Animal feeds

Twelve hours before surgery, food was withheld to avoid the hazards of aspiration after anaesthesia induction. Free access to water was provided until one hour prior to the surgery and after complete recovery from anaesthesia.

4.5 Investigational veterinary (IVP) and control products (CP)

- IVP: S (+) ketamine 2 mg/kg BW intravenously (IV) (Keta-S ad us. vet., Injektionslösung Dr. E. Graeub AG, Bern, Switzerland)

- CP: racemic ketamine 4 mg/kg BW intravenously (IV) Ketasol-100 ad us. vet., Injektionslösung Dr. E. Graeub AG, Bern, Switzerland

4.6 Treatments: Anaesthesia and surgery management

4.6.1 Preparation and pre-medication

The patients were admitted to the clinic the day of surgery. They were systematically examined to assess preoperative physiological parameters and ASA classification. Their inclusion or exclusion from the study was determined. Patients that met the inclusion criteria were then transferred to the preparation room next to the surgery room.

The study animals in both groups were first filmed for assessment of normal behaviour in a foreign environment (preparation area) for a period of 5 minutes without collar and for 5 minutes with collar. Once ready each animal received an intramuscular (hind limb muscle) injection of 450 μg/m^2 body surface area (BSA) of medetomidine and 0.2 mg/kg body weight (BW) butorphanol, mixed in the same syringe. Ten minutes after injection of sedative agents, the dogs were examined to assess the following parameters: respiratory frequency (RR), heart rate (HR), mucous membrane colour (MMC) and capillary refill time (CRT).

When sedation was achieved, 20 - 30 minutes after medetomidine and butorphanol administration, an intravenous catheter was placed on the right or left cephalic vein.

4.6.2 Anaesthesia induction

After sedation, application of a venous catheter and preparation of the operative site (clipping of hairs, first alternated povidone-iodine and alcohol application), the dogs were moved by the investigator and the supporting person on a surgery table in lateral recumbency until ketamine (blind randomised) was administrated.

Thirty minutes after administration of medetomidine and butorphanol, the dogs received 2 mg per kg body weight of S (+) ketamine (s-ket group) or 4 mg/kg BW of racemic ketamine (rac-ket group). After anaesthesia was induced, the animals were intubated with a cuffed endotracheal tube after applying 2% lidocaine on each side of the larynx. A 500 mL Ambu-bag was connected to the endotracheal tube via its one way valve and a constant oxygen flow (0,5-2 l/min, depending on the size of the dog) was insufflated through the distal port of its self inflating chamber during the whole surgery to ensure continuous oxygen delivery to the patient. Should apnoea have occurred, the dogs were ventilated using the Ambu-bag. The study animals were then placed in dorsal recumbency on the surgery table and prepared for surgery (a standard antiseptic

procedure was used). The scrotum was desensitised using 1 mg/kg BW of lidocaine prior surgical incision.

Once the patient was positioned and the skin prepared, the investigator started the last phase of surgery preparation, including draping and preparation of the surgical instruments and material. Meanwhile, the supporting person (a veterinarian) applied the following anaesthesia monitoring instruments:

- ECG, Pulsoxymeter (SpO_2), arterial blood pressure (DAP, MAD, SAP): HP 78352C, Hewlett Packard Corporation
- Capnograph ($EtCO_2$): Capnomac ™ II AGM-123, Datex Instrumentarium Corporation

4.6.3 Anaesthesia maintenance and surgery

Once ready, approximately 5 minutes after ketamine administration, surgery began (standard orchiectomy). Physiological parameters were monitored permanently during anaesthesia. Respiratory frequency, HR, oxygen saturation (SpO2), SAP, MAP, DAP, mucous membrane colour (MMC) and CRT were recorded every 5 minutes. Presence/absence of the eyelid reflex was evaluated by gently palpating the medial cantus of the eye. Should the patient have moved or displayed signs of awakening from anaesthesia (tachypnea, increased jaw tone, brisk eyelid reflex), an IV bolus of 1 mg/kg BW S (+) ketamine or 2 mg/kg BW was given for patients in the s-ket group or r-ket group, respectively. An infusion of 10 ml/kg/h of Ringer's lactated solution was delivered during the whole anaesthesia through the cephalic vein.

Antibiotics (amoxicillin and clavulanic acid, Synulox ® Suspension Pfizer ad us. vet., Injektionssuspension 8.75 mg/kg BW, SC) and long term analgesics (carprofen, Rimadyl ® Pfizer ad us. vet., Injektionslösung 4 mg/kg BW, IV) were administered after surgery.

4.6.4 Recovery phase and postoperative care

Sixty minutes after ketamine administration, atipamezole (Antisedan ® Pfizer ad us vet., at the at the dose 2250 $\mu g/m^2$ (IM) (= time point 0) was administered. Animal observation and video recording were immediately started to assess behaviour and sedation, and continued for an hour. At time points 0, 10, 20, 30 and 60 assessment sedation and behaviour were performed. In addition, HR, pulse character RR, MMC and rectal temperature were recorded 0, 30 and 60 minutes after atipamezole administration.

Sixty minutes after atipamezole administration, video recording stopped and the study was concluded. The video tape recordings were used to re-evaluate particular cases regarding the behavioural and sedation parameters.

4.6.5 Disposal of study animals

During the same day of the surgery, all animals were returned to their owner respectively to the animal shelter.

4.7 Handling of records

4.7.1 Owner consent forms

Owner consent forms (Annex A) were filled out and signed by the owner the day of the trial, before the beginning of the pre-operatory examination.

4.7.2 Video Records.

Each animals was filmed by the investigator:

- Pre-operatory periods of 10 minutes were filmed (first 5 minutes without collar and then 5 minutes with collar).
- Recovery period: the whole period between atipamezole administration (=time point 0) and the complete recovery (=time point 60) was filmed

4.7.3 Case Reports Forms (CRF)

4.7.3.1 Pre-admission examination (page 1 of 3): baseline clinical data

- Date, CRF Number, group, ASA classification, patient data (name, owner, breed, age)
- Pre-anaesthesia exam (pre-admission data measurement)
- Body condition: weight, hydration status, body temperature)
- Cardiovascular and respiratory system: respiratory rate, heart rate, mucous membrane colour, capillary refill time, pulse character, thoracic auscultation

Other systems and complementary information:

- Concomitant drugs (yes/no)
- Inclusion (yes/no)
- Venous catheter (right/left)
- Sedation: time, medetomidine and butorphanol doses, observations, heart rate, respiratory rate, pulse character, capillary refill time, mucous membrane colour

4.7.3.2 Anaesthesia (page 2 of 3): anaesthesia monitoring (intraoperative data)

- Anaesthesia induction: time, ketamine (A or B, dose), observations
- Intubation: time, tubus size
- Constant anaesthesia monitoring: time, O2 Flow, HR, SAP, DAP, MAP, RR, SpO2 %, MMC, CRT, End tidal CO_2, eyelid reflex (+/-), body position, observations
- Surgery beginning time, surgery ending time
- Application of lidocaine, carprofen and atipamezole doses
- Atipamezole injection time (=Time point 0)
- Ketamine re-dosing (Yes/no, dose)

4.7.3.3 Recovery (page 3 of 3): Post operative data measurement

During recovery of the first dog (CRF 1), the degree of sedation and the behaviour rapidly changed within the first 20 minutes. It was decided, that more measurements would be necessary during this period. Therefore the case report form was changed and future measurements (sedation, behaviour, vocalisations, muscle tone, eyes movements, tongue movements and salivation) were recorded for time points 0, 10, 20, 30 and 60. The case report forms were adapted accordingly. The records of cardiovascular data at time points 0, 30 and 60 remained unaltered. Extubation time was also recorded and added on the case report form for anaesthesia monitoring.

- Clinical examination at time point 0, 30, 60 minutes **after reversal of anaesthesia** (RR, mucous membrane colour, HR, capillary refill time, pulse character). Body temperature was assessed at time points 0 ad 60.
- Assessment of sedation at time points 0, 10, 20, 30, 60 **after** atipamezole administration (Table 2).

Table 2: Sedation scores

No sedation	0	Fully alert, reacting to noises
Poor sedation	1	Alert but with somnolence, reacting to noises
Moderate sedation	2	Drowsy but occasionally restless
Deep sedation	3	Sleeping comfortable

- Evaluation of **behaviour** at time points 0, 10, 20, 30, 60 minutes **after** atipamezole administration, scaled on the basis of the particular case and the clinical judgement of the investigator, as follows:

  - Clinical observations: eyes (myosis, mydriasis, third eye lid prolapsed, eye rolled ventrally, nystagmus) muscles (relaxation, tone increased, stiffness, tremor), tongue movements, presence or absence of salivation.
  - Other clinical signs not listed on the form, but relevant to the investigator were recorded also.
  - Time to sternal recumbency, time to standing position.

5. Data analysis

5.1 Statistical methods

NCSS 2004 (Kaysville, Utah, USA) software package was used to perform the statistical evaluation.

- All data were analysed for normal distribution with the Shapiro-Wilk W test.
- The significance of difference between groups for parametric data was assessed with Student's t-tests or with ANOVA for data with more than two levels of significance.
- Non-parametric variables were evaluated using Mann-Whitney U or Kurskal-Wallis tests.
- Proportions were analysed with Chi-squared tests.
- For all tests, overall $P < 0.05$ was considered the minimum level of statistical significance.
- A trend towards a statistical significance was defined as $P = 0.1$.
- Parametric data are presented as mean ± SD and non-parametric data are presented as mean, median and range and/or box plots.

6. Results

6.1 Animal details (population description): Pre-anaesthesia data

6.1.1 Animal species and breeds

The dog breeds, included in Table 3, were included into the study.

Table 3: Breeds included in the study

Breed	Frequency	Percentage
American Staffordshire	2	2.4
Border Collie	4	9.75
Chinese crested hairless	1	2.4
Dalmatian	1	2.4
Dogo argentine	1	2.4
Épagneul bleu de Picardie	1	2.4
Labrador	3	7.3
Lhassa Apso	1	2.4
Pitt bull	1	2.4
Rottweiler	1	2.4
Shar pei	2	4.9
Shi-Tzu	1	2.4
Swiss white shepard	1	2.4
Terrier du Tibet	1	2.4

Breed	Frequency	Percentage
American Staffordshire	2	2.4
Border Collie	4	9.75
Chinese crested hairless	1	2.4
Westhighland Terrier		2.4
Mixed breed	22	53.7
Total	41	100.0

53.7 % of the dogs included in our study were by mixed breed's dogs and the frequencies along our randomisation list showed us that no relevant statistical difference was present.

6.1.2 Animal age

Animal age was known in 35 of 41 dogs. For 2 dogs from animal shelter and 4 dogs from private owner, the exact age was unknown. The other dogs had a mean age of 2.1 years (SD ± 1.5) with a minimum of 0.5 and a maximum of 7 years (Fig.13).

The differences between the two groups were statistically irrelevant (p= 0.72).

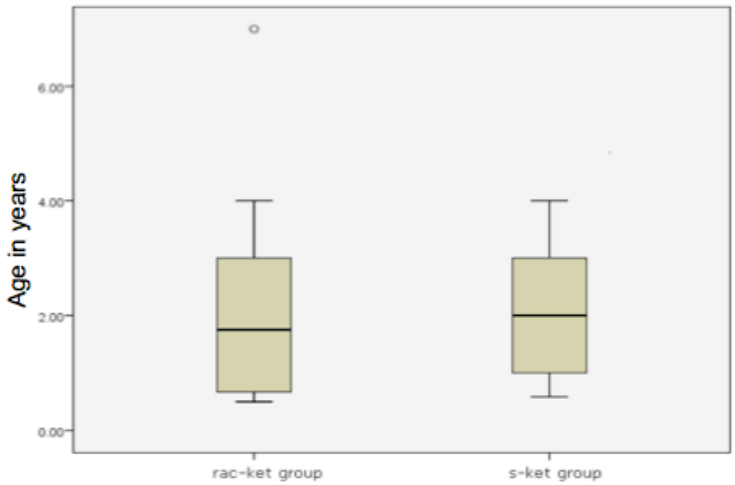


Figure 13: Animal age in years

6.1.3 Animal weight

Dogs had a mean body weight of 24.0 kg (SD ± 9.9 kg), Fig.14.

The differences between the two groups were statistically irrelevant (p=0.81).

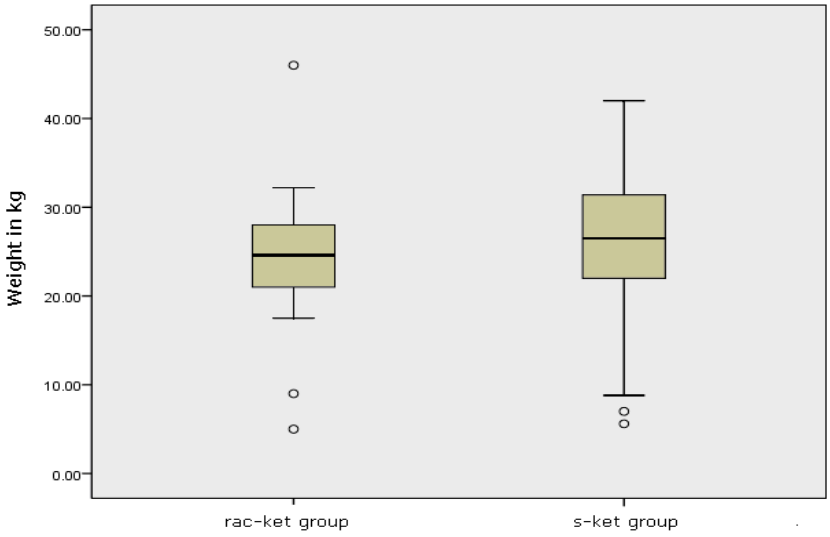


Figure 14: Animal weight in kg bodyweight

6.1.4 Heart rate (HR)

Dogs (n=41) had, before sedation, a pre-operative mean heart rate of 77.7 beats / min (SD ± 17.8) with a minimum of 44.0 and a maximum of 120.0 beats/min.
There was no statistical relevant difference between both groups (p= 0.86).

6.1.5 Pulse character

Three different categories (Table 4) were used in this study to describe the pulse character of the *arteria femoralis*: weak, normal and strong. Assessed by the principal investigator.

Table 4: Pre-operative pulse characters

	rac-ket group	s-ket group
Weak	0 (0.0%)	0 (0.0%)
Normal	4 (20%)	4 (19%)
Strong	16 (80%)	17 (81%)
Total	20 (100%)	21 (100%)

No statistical relevant differences were found between both groups (p = 0.94).

6.1.6 Respiratory rate (RR)

19 of 41 dogs were panting during pre-anaesthesia clinical examination. That's why respiratory rates were grouped into 3 categorical groups (Table 5) :

- Category 1 included values between 1 and 30 breath/min;
- Category 2 included values between 31 and 50 breath/min;
- Category 3 included values above 50 breaths/min or panting;

Table 5: Pre-operative respiratory rates

	rac-ket group	**s-ket group**
Category 1	7 (35%)	9 (42.9%)
Category 2	1 (5.0%)	5 (23.8%)
Category 3	12 (60.0%)	7 (33.3%)
Total	20 (100%)	21 (100%)

No statistical relevant differences were found between both groups (p = 0.12).

6.1.7 Mucous membrane colour (MMC)

Three different categories of MMC were used in the study: pale, pink (normal reference) and red. During the pre-operative examination just one dog in rac-ket group showed red mucous membranes, all others had pink mucous membranes.

No statistical relevant differences were found between both groups (p = 0.29).

6.1.8 Body temperature, capillary refill time (CRT), body condition, hydratation status and thoracic auscultation

Body temperature was considered normal in 40 of 41 dogs. In one dog (CRF 12), belonging to rac-ket group, body temperature was elevated (39.6°C). As all other clinical parameters were normal and, as the dog didn't show any history of sickness, it was decided to include the dog in the study.

One dog (CRF 10) belonging to rac-ket group had a slightly poor body condition; all others were considered to be of normal constitution. No abnormal thoracic auscultation finding was detected. Hydratation status was good in all dogs. Subjective evaluation of capillary refill time was considered adequate in all dogs: all values ranged between 1 and 2 seconds.

In conclusion, both groups were considered homogenous for pre-operative data.

6.2 Intra operative data measurement

The population of our study decreased from 41 to 37 dogs. Four dogs (CRF 2, 3, 15a and 22) all belonging to s-ket group were excluded from the study because supplemental ketamine was necessary in order to terminate elective surgery under good conditions.

6.2.1 Heart Rate (HR)

Heart rate was obtained in all dogs. Overall values were not significantly different between groups, although a trend was observed (p = 0.087) for higher heart rates in rac-ket group (80.5 beats/min [45 -138]) compared with s-ket group (74 beats/min [35 - 134]).

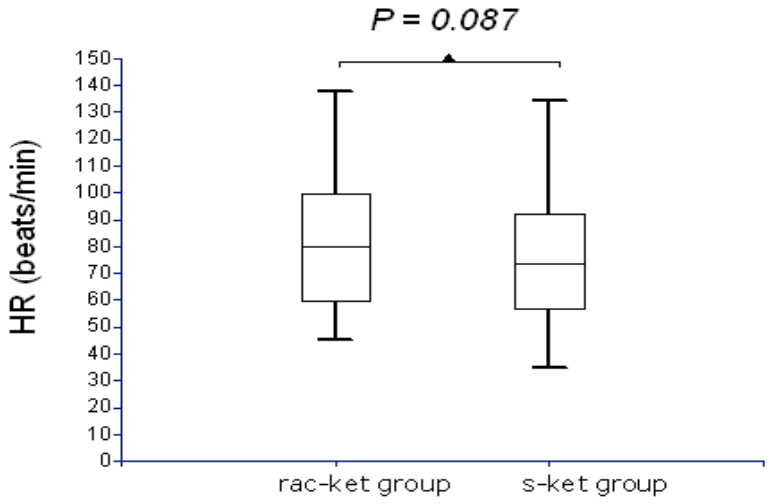


Fig. 15: Mean intraoperative heart rates averaged over time

Over time, heart rates slightly decreased progressively in both groups. Values for heart rates were similar between groups, but a trend (p = 0.06) for higher heart rates in rac-ket group compared with s-ket group was found 25 minutes after ketamine administration (indicated on Fig.16 with ＃).

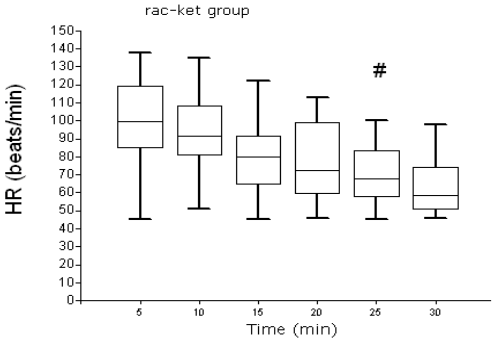


Fig. 16a: Mean Intra-operative heart rate values over time in rac-ket group

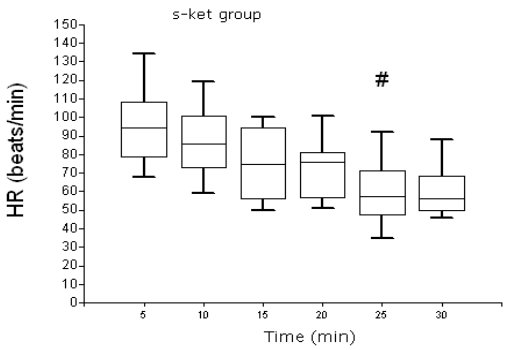


Fig. 16b: Mean Intra-operative heart rate values over time in s-ket group

6.2.2 Respiratory Rate (RR)

Respiratory rate was monitored in all dogs. There were no statistically significant differences (p = 0.22) between both randomisation groups (rac-ket group: 8 breaths/min [2-28]; s-ket group: 8 breaths/min [2-25].

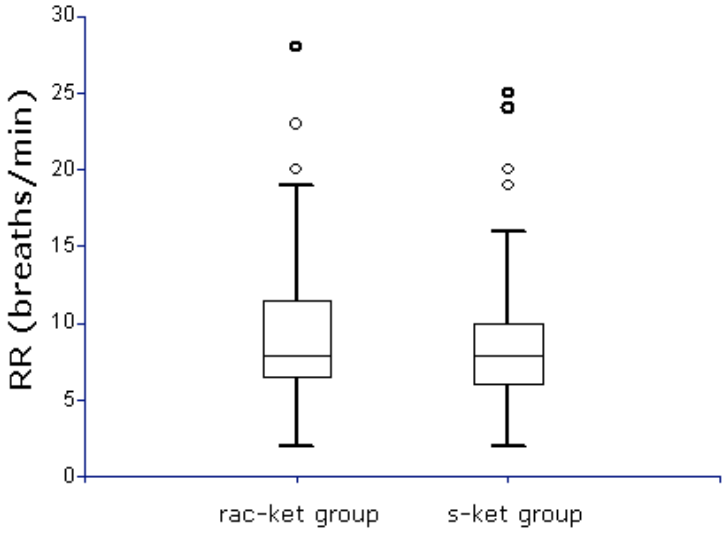


Fig. 17: Mean intra-operative respiratory rate values

Shortly after ketamine administration, 3 dogs (CRF 5, 7 and 9) from rac-ket group showed apnoea that lasted for 30-40 seconds. In dogs CFR 5 and 7, spontaneous ventilation was reassumed after that short period of time and no assisted ventilation was required. In dog CFR 9 manual ventilation was necessary until spontaneous ventilation was restored at 5 minutes after ketamine administration.

15 minutes after anaesthesia induction, respiratory rates increased progressively in both groups as evaluated by visual examination of the box plots. No differences were found between groups at any evaluated time point ($p > 0.05$ for all time points).

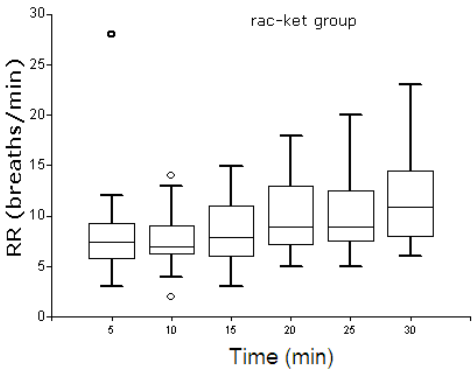


Fig. 18a: Mean intra-operative respiratory rate values over time in rac-ket group

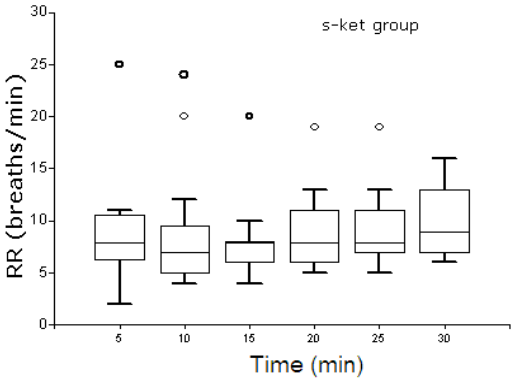


Figure 18b: Mean intra-operative respiratory rate values over time in s-ke group

6.2.3 End tidal CO_2 ($EtCO_2$)

End tidal CO_2 was obtained in all dogs. Values were significantly different (p = 0.006) between groups (rac-ket group = 40 mm Hg [15 - 88]; s-ket group = 36.5 mm Hg [8 - 58]).

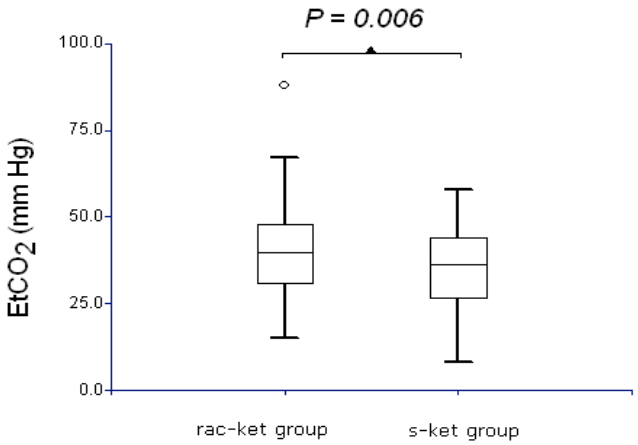


Fig. 19: Box plots for mean $EtCO_2$ averaged over time

Over time, results for $EtCO_2$ were different between both groups: dogs in rac-ket group presented higher EtCO2 values than dogs in s-ket group during the first 10 minutes after anaesthesia induction (t=5 and t=10, p = 0.03 for both time points, indicated with on Fig. 20 with the sign *).

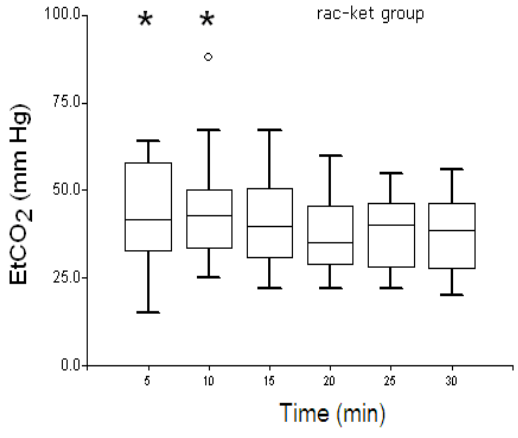


Fig. 20a: $EtCO_2$ over time in rac-ket group

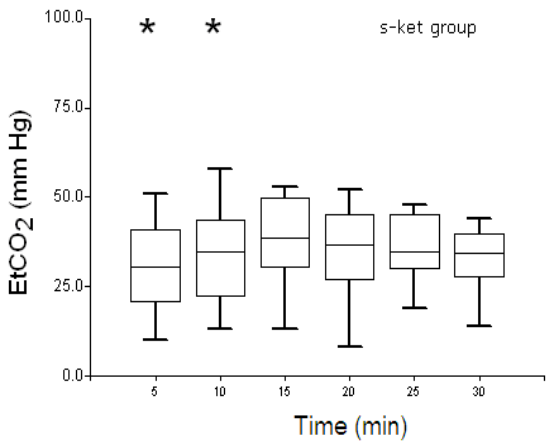


Fig 20b: $EtCO_2$ over time in s-ket group

6.2.4 Haemoglobin oxygen saturation (SpO_2)

Values for SpO_2 were not significantly different (p = 0.69) between groups (median: 99% [81 -100] for both groups). Only 4 and 2 isolated readings below 90% were observed in rac-ket and s-ket group, respectively.

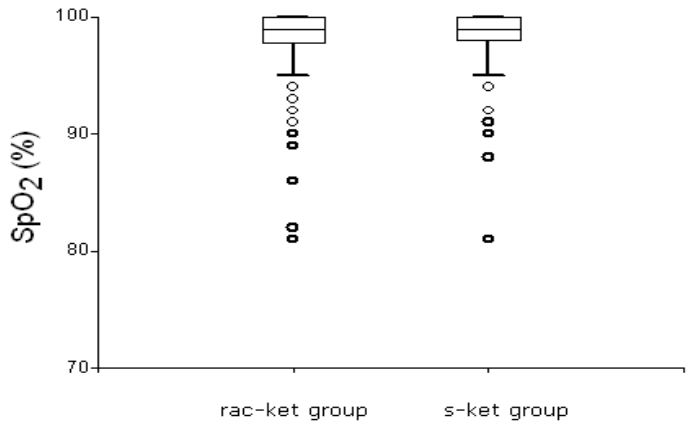


Fig. 21: Box plots for mean) SpO_2 averaged over time

6.2.5 Indirect arterial blood pressure (DAP, MAP, SAP)

Indirect arterial blood pressure was obtained in 14 out of 20 dogs in rac-ket group and in 16 out of 17 dogs in s-ket group.

Values for DAP were not significantly different (p=0.43) between groups (rac-ket group: median 84 mm Hg [30-174]; s-ket group, median: 86.5 mm Hg [14 -116]).

Values for MAP were not significantly different (p=0.47) between groups (rac-ket group: median 100 mm Hg [42 -193]; s-ket group, median: 99 mm Hg [42 -140]).

Values for SAP were not significantly different (p=0.82) between groups (rac-ket group: median 134 mm Hg [68 - 227]; s-ket group: median 133 mm Hg [87-174]).

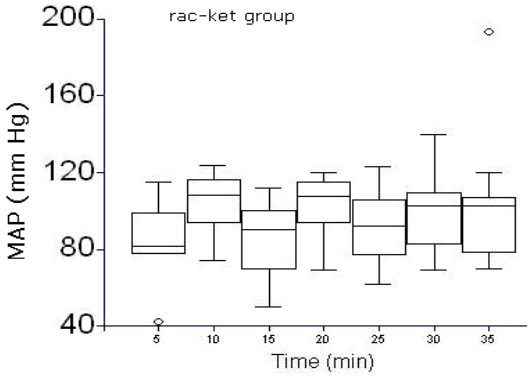


Fig. 22a: Mean MAP over time in rac-ket group

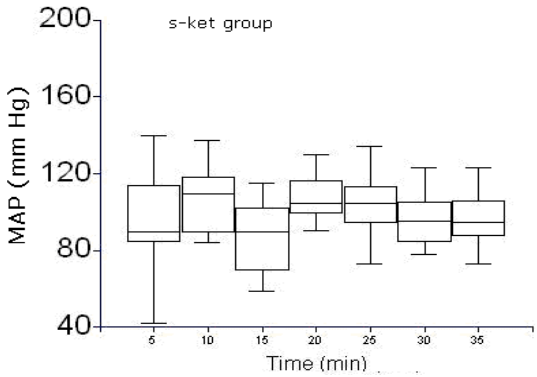


Fig. 22b: Mean MAP over time in s-ket group

6.2.6 Capillary refill time

Subjective evaluation of capillary refill time was considered adequate in all dogs. All values ranged between 1 and 2.5 seconds.

6.2.7 Eyelid reflex

There was a statistically significant difference in eyelid reflexes between groups (p=0.006) with more dogs showing positive eyelid reflexes in s-ket group than in rac-ket group. However, differences were not statistically significant when data were plotted over time and only a trend (p=0.09).for significant difference was found at 10 min (indicated with # on Fig. 23a and Fig.23b).

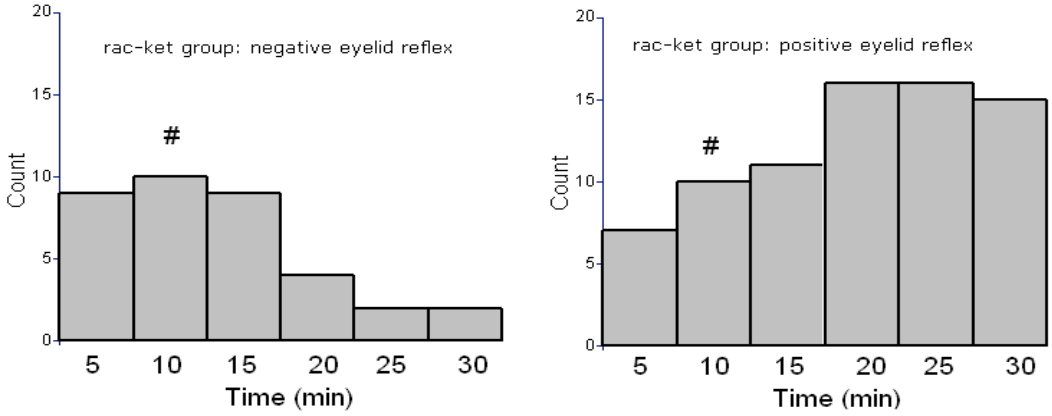


Fig. 23a: Eyelid reflexes over time in rac-ket group

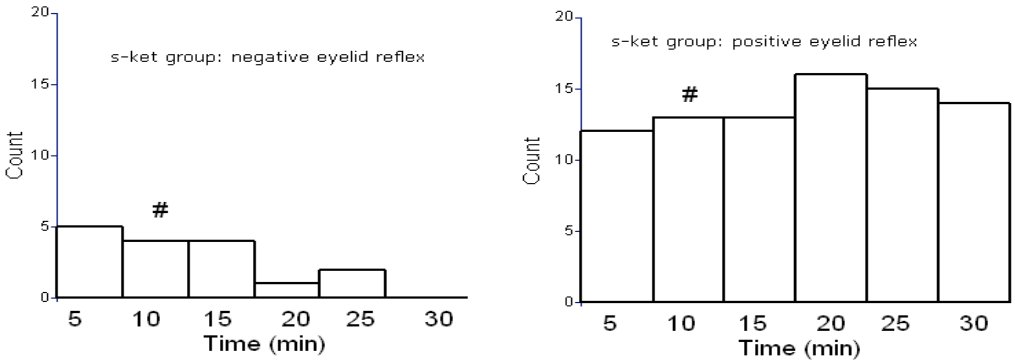


Fig. 23b: Eyelid reflexes over time in s-ket group

6.2.8 Adverse events during anaesthesia induction and maintenance until reversal with atipamezole

- CRF 2, CRF 3, CRF 15a: all belonging to the s-ket group needed supplemental bolus of ketamine (re-dosing). Those patients were excluded from the study.
- CRF 5: belonging to the rac-ket group. 60 minutes after atipamezole administration the dog had diarrhoea once. No supplementary medication was necessary. The dog returned to the animal shelter.
- CRF 6: belonging to the s-ket group, the patient was excluded for the evaluation of post-operative data because approximately 30 minutes after anaesthesia induction it started to show seizures-like episodes (only up-down head movements) triggered by moving the dog from the operating room to the recovery room. Seizures were successfully treated with 5 mg intravenous diazepam, physiologic saline solution and nasal oxygen insufflations. Half an hour later the patient was re-sedated with 0.02 mg/kg acepromazine and 0.2 mg/kg butorphanol IV. The dog recovered without sequelae. This adverse event was announced to Swissmedic in line with the pharmacovigilance System of Dr. E. Graeub AG.
- CRF 18: belonging to the rac-ket group was excluded for the evaluation of post-operative data because 31 minutes after anaesthesia induction, it started to show seizures-like episodes (only short up-down head movements), triggered by moving the dog from operating room to recovery room. The dog was treated with 5 mg intravenous diazepam. The dog recovered without sequelae.
- CRF 22 belonging to the rac-ket group needed supplemental bolus of ketamine (re-dosing). This patient was excluded from the study. The dog recovered without sequelae.

All dogs recovered well and safely and were returned save at home or to the local animal shelter the same day of the surgery.

6.3 Recovery

Overall, all dogs recovered well from anaesthesia and returned home at the end of the experiment. Two dogs CRF 6, belonging to s-ket group and CRF 18 belonging to rac-ket group were excluded from evaluation of post-operative data because they had seizures-like episodes approximately 30 minutes after ketamine administration and received supplemental medication that influenced the anaesthesia.

No statistically significant differences between groups regarding mucous membrane colour, capillary refill time, body temperature and pulse quality (Table 6) were detected during recovery. Thoracic auscultation was considered normal in all dogs.

Table 6: Pulse quality at recovery

Pulse		rac-ket group n= 19	s-ket group n=19	p-value
T=0	Weak	0 (0.0%)	0 (0.0%)	0.68
	Normal	12 (63.2%)	9 (56.3%)	
	Strong	7 (36.8%)	7 (43.7%)	
T=30	Weak	0 (0.0%)	0 (0.0%)	0.75
	Normal	14 (73.7%)	11 (68.8%)	
	Strong	5 (26.3%)	5 (31.2%)	
T=60	Weak	0 0.0%)	0 (0.0%)	0.97
	Normal	12 (63.2%)	10 (62.5%)	
	Strong	7 (36.8%)	6 (37.5%)	

6.3.1 Heart Rate

On average, over time, there was a trend ($p = 0.056$) for higher heart rate values in dogs allocated to rac-ket group (median 64 beats/min [28-140]) compared to those in s-ket group (median 60 beats/min [36-120]).

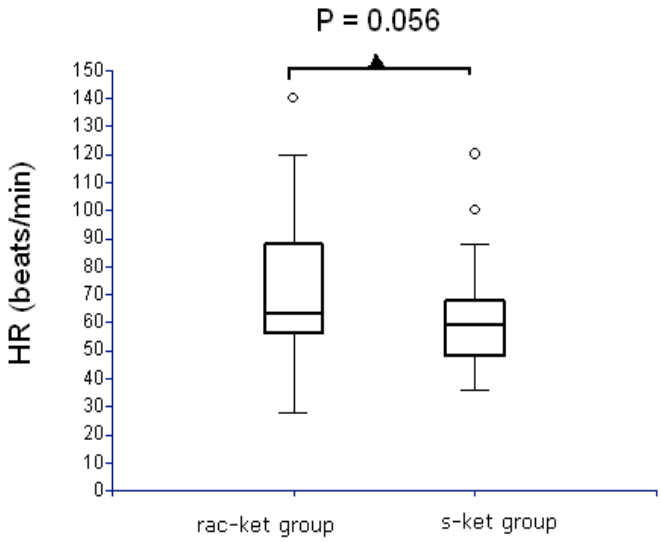


Fig. 25: Box plots of post-operative median heart rate values averaged over time

Over time, both groups showed a significant increase in HR values (p 0.001), after atipamezolde injection, but no significant differences were evident between groups at each time point.

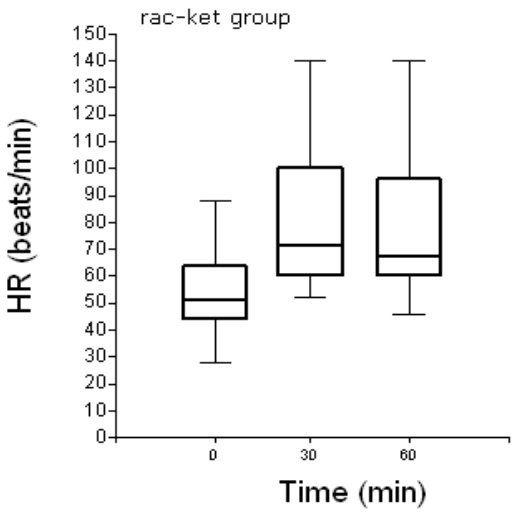


Fig. 26a: Post-operative heart rate values over time in rac-ket group after atipamezole administration

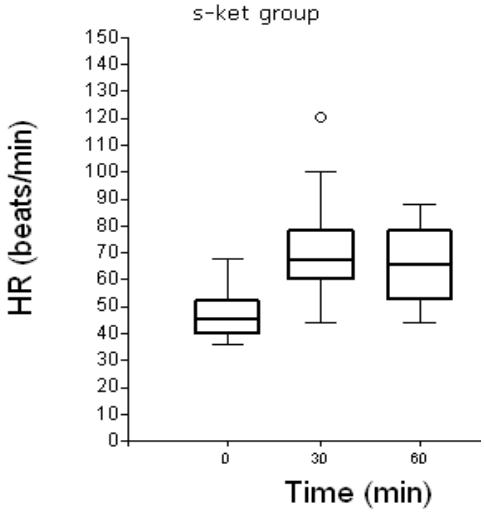


Fig. 26b: Post-operative heart rate values over time in s-ket group after atipamezole administration

6.3.2 Respiratory rate

Overall, both groups had similar (p = 0.33) respiratory rate values during the recovery period (rac-ket group: median: 5 breaths/min [2-32]; s-ket group median: 5 breaths/min [3-32]). Over time, RR values significantly (p = 0.001) decreased in both groups. No significant differences were detected between groups at each evaluated time point.

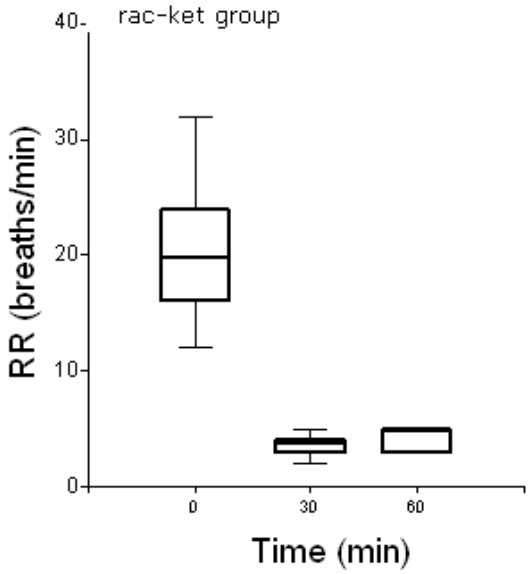


Fig. 27a: Mean Post-operative respiratory rates over time in rac-ket group

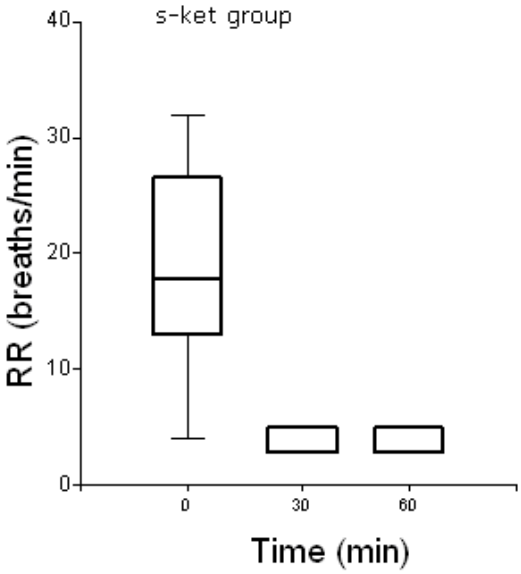


Fig. 27b: Mean Post-operative respiratory rates over time in s-ket group

6.3.3 Sedation

Sedation scores were taken just after the administration of atipamezole (t=0), and 10 (t=10), 20 (t=20), 30 (t=30) and 60 minutes (t=60) after atipamezole administration.

Overall distributions of sedation values were comparable between groups (p = 0.97). Similarly, there were no statistically significant differences in regards to sedation values at each evaluated time point.

Table 7a: Sedation scores at time just after atipamezole administration (=time point 0)

	No sedation	Poor sedation	Moderate sedation	Deep sedation	Total	p-value
rac-ket	0 (0.0%)	2 (10.5%)	1 (5.3%)	16 (84.2%)	19 (100%)	0.72
s-ket	0 (0.0%)	2 (12.5%)	2 (12.5%)	12 (75.0%)	16 (100%)	
Total	0 (0.0%)	4 (11.4%)	3 (8.6%)	28 (80.0%)	35 (100%)	

Table 7b: Sedation scores at 10 minutes after atipamezole administration (= time point 10)

	No sedation	Poor sedation	Moderate sedation	Deep sedation	Total	p-value
rac-ket	9 (47.4%)	6 (31.6%)	4 (21.1%)	0 (0.0%)	19 (100%)	0.71
s-ket	8 (50.0%)	4 (25.0%)	3 (18.8%)	1 (6.3%)	16 (100%)	
Total	17 (48.6%)	10 (28.6%)	7 (20.0%)	1 (2.9%)	35 (100%)	

Table 7c: Sedation scores at 20 minutes after atipamezole administration (= time point 20)

	No sedation	Poor sedation	Moderate sedation	Deep sedation	Total	p-value
rac-ket	16 (84.2%)	3 (15.8%)	0 (0.0%)	0 (0.0%)	19 (100%)	0.78
s-ket	14 (87.5%)	2 (12.5%)	0 (0.0%)	0 (0.0%)	16 (100%)	
Total	30 (85.7%)	5 (14.3%)	0 (0.0%)	0 (0.0%)	35 (100%)	

Table 7d: Sedation scores at 30 minutes after atipamezole administration (= time point 30)

	No sedation	Poor sedation	Moderate sedation	Deep sedation	Total	p-value
rac-ket	19 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	19 (100%)	
s-ket	16 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	16 (100%)	
Total	35 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	35 (100%)	

Table 7e: Sedation scores at 60 minutes after atipamezole administration (= time point 60)

	No sedation	Poor sedation	Moderate sedation	Deep sedation	Total	p-value
rac-ket	19 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	19 (100%)	
s-ket	16 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	16 (100%)	
Total	35 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	35 (100%)	

Sedation scores over time:

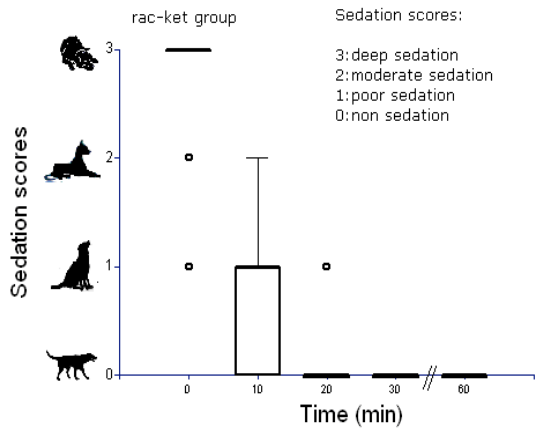


Fig. 28a: Sedation scores over time in rac-ket group

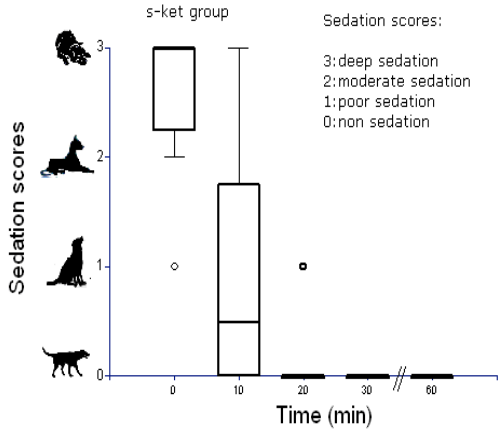


Fig 28b: Sedation scores over time in s-ket group

6.3.4 Times to sternal and standing position

There were no statistical differences in regard to time to first movement (p=0.09), time to sternal position (p=0.36) and time to sitting position (p=0.94) between both groups (Table 8).

Table 8: Mean times to first movement, sternal position and sitting position and ranges.

		N	overall	rac-ket group	s-ket group	p-value
Time to first movement	Min	35	3.9 [0-13.8]	3.7 [0-8]	5.35 [0.2-13.8]	0.09
Time to sternal position	Min	35	5.8 [0.8-27.9]	5.7 [1.2-24.3]	6.95 [0.8-27.9]	0.36
Time to sitting position*	Min	33	11.5 [1.2-47.8]	11.3 [1.2-47.8]	11.9 [5.3-28]	0.94

*In the case that dogs went directly from sternal to standing position, without ever staying in a sitting position, time to achieve standing position was considered and included for calculation of "time to sitting position".

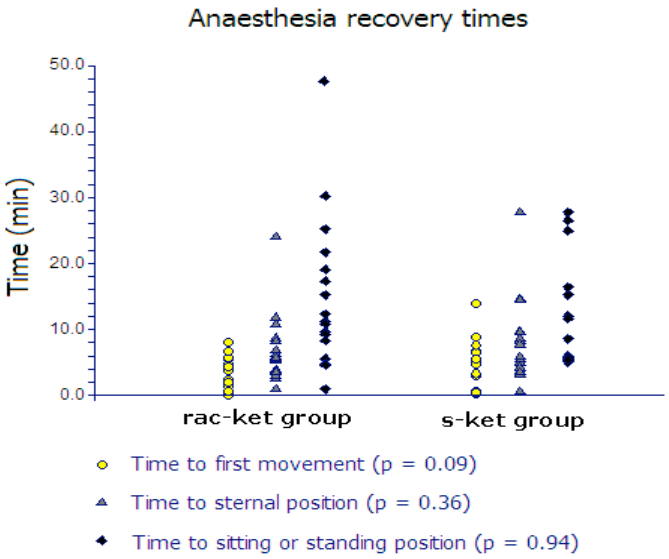


Fig. 29: Mean anaesthesia recovery times

6.3.5 Behaviour

Overall, distributions of behaviour scores were not significantly different between groups (p = 0.57). Similarly, there were no statistically significant differences in regards to behaviour scores at each evaluated time point (Table 9).

Table 9a: Behavioural scores just before atipamezole administration (=time point 0)

	Normal	Minor changes	Moderate changes	Severe changes	Total	p-value
rac-ket	17 (89.5%)	1 (5.3%)	1 (5.3%)	0 (0.0%)	19 (100%)	0.56
s-ket	14 (87.5%)	1 (6.3%)	0 (0.0%)	1 (6.3%)	16 (100%)	
Total	31 (88.6%)	2 (5.7%)	1 (2.9%)	1 (2.9%)	35 (100%)	

Table 9b: Behavioural scores 10 minutes just before atipamezole administration (= time point 10)

	Normal	Minor changes	Moderate changes	Severe changes	Total	p-value
rac-ket	1 (5.3%)	11 (57.9%)	7 (36.8%)	0 (0.0%)	19 (100%)	0.32
s-ket	3 (18.8%)	6 (37.5%)	7 (43.8%)	0 (0.0%)	16 (100%)	
Total	4 (11.4%)	17 (48.6%)	14 (40.0%)	0 (0.0%)	35 (100%)	

Table 9c: Behavioural scores 20 minutes just before atipamezole administration (= time point 20)

	Normal	Minor changes	Moderate changes	Severe changes	Total	p-value
rac-ket	3 (15.8%)	13 (68.4%)	3 (15.8%)	0 (0.0%)	19 (100%)	0.55
s-ket	3 (18.8%)	8 (50.0%)	4 (25.0%)	1 (6.3%)	16 (100%)	
Total	6 (17.1%)	21 (60.0%)	7 (20.0%)	1 (2.9%)	35 (100%)	

Table 9d: Behavioural scores 30 minutes just before atipamezole administration (= time point 30)

	Normal	Minor changes	Moderate changes	Severe changes	Total	p-value
rac-ket	6 (31.6%)	11 (57.9%)	1 (5.3%)	1 (5.3%)	19 (100%)	0.81
s-ket	6 (37.5%)	9 (56.3%)	0 (0.0%)	1 (6.3%)	16 (100%)	
Total	12 (34.3%)	20 (57.1%)	1 (2.9%)	2 (5.7%)	35 (100%)	

7. Discussion

Early human studies of ketamine isomers (White *et al.*, 1980) appeared to demonstrate that the S (+) ketamine produces less psychic emergence reactions than the R (-) ketamine or the racemic mixture. Moreover, the S (+) ketamine in humans, horses and cats has proven advantageous by reaching an identical plane of anaesthesia with half of the racemic dose.

7.1 Pre-anaesthesia data

All dogs were considered healthy on the basis of a pre-admission examination performed pre-operatively. In one dog, body temperature was elevated, but as all other clinical parameters were normal, it was included in the study. The elevated temperature had no relevant negative impact on the anaesthesia procedure. Both groups were considered homogenous for the pre-operative data.

7.2 Sedation

In the present study the dogs were sedated by using the combination of an alfa-2 adrenoceptor agonist (medetomidine) and butorphanol.

Although the coadministration of drugs might have a significant impact on metabolism of ketamine enantiomers in cats and dogs (Balmer *et al.,* 2008; Larenza *et al*. 2008; Baumgartner *et al*., 2002), the combination of medetomidine with butorphanol is clearly indicated as stated in the study of Ko *et al.* (2000).

7.3 Induction with Ketamine

Thirty minutes after administration of medetomidine and butorphanol, both ketamine solutions – racemic ketamine and S (+) ketamine – were administered intravenously.

In the current study used a dose of 2 mg per kg BW of S (+) ketamine (s-ket group) and 4 mg/kg BW of racemic ketamine (rac-ket group). These doses were considered equipotent and chosen based on preliminary data collected in sedated dogs and on data of other species. Similar doses have been successfully applied to cats (Larenza *et al.,* 2008; Balmer *et al.,* 2005; Baumgartner *et al.,* 2002; Stelter *et al.*, 2001) and horses (Larenza *et al.*, 2007).

The dose rates chosen here are in contrast with the study of Duque *et al.* (2008), where a dose reduction of 50% of S (+) ketamine in comparison to racemic ketamine didn't suggest equipotent anaesthetic effects in dogs. However in this study the authors did only apply racemic vs. S (+) ketamine during their trial (a) without the combination of other drugs and also (b) with clearly higher doses of ketamine (9 mg/kg for the racemic ketamine and respectively 6 mg per kg BW for the S (+) ketamine. As the sole use of

ketamine is causing muscular spasms it is unethical to use it in this way in clinical cases.

7.4 Intra-operative data

Heart rate. Heart rates were not significantly different between both groups, but dogs in rac-ket group had slightly higher heart rates than dogs receiving S (+) ketamine. Over time, heart rates slightly decreased in both groups. Graf *et al.* (1995) reported, using the isolated perfused heart model, that S (+) ketamine is less depressant compared to racemic and isomeric R (-) ketamine.

Intra-operative values for arterial blood pressure and capillary refill time were within normal ranges in all dogs and no differences were detected between both groups.

Respiratory function. After ketamine administration, 3 dogs from rac-ket group showed apnoea. In one dog manual ventilation was necessary. Over time, respiratory rates progressively increased in both groups. Ketamine is one of the most reliable anaesthetic agent for anaesthesia induction and maintenance, since at clinically useful dose rates it does not or only mildly impair respiration (Paix *et al.*, 2005; Werner *et al.*, 1997). The transient apnoea induced by ketamine appears to be dose dependent. At higher doses, respiration is characterised by an apneustic, shallow, and irregular pattern (Sears, 1971). Severe depression or arrest with over dosage has been described in humans and cats (Kopman, 1972; Child *et al.*, 1972; Bree *et al.*, 1972). In the present report the fact that only in the racemic ketamine group apnoea occurred might suggest, that the dose rate used was slightly more potent than the one of the S-ketamine group. This is also supported by the fact, that end tidal carbon dioxide concentration was significantly higher in rac-ket group than in s-ket group during the first 10 minutes after anaesthesia induction . Even if all values were within acceptable ranges, this result could be an indication for better alveolar ventilation in s-ket group, at the dose ranges tested.

Metabolism/duration of action of ketamine. After surgery 3 dogs from S-ket group (S (+) ketamine) were excluded from the study because ketamine re-dosing (supplemental bolus) was necessary to complete the routine orchiectomy. Differences in ketamine action could originate from the pharmacokinetics (absorption, bioavaibility, distribution, metabolism and elimination), from pharmacodynamics (number of receptors, subtypes of receptors, affinity to receptors) or both (Duque et al., 2008). In the case of S-ketamine it is most likely a pharmacokinetic phenomenon. When administered as a sole agent in men and dogs, the plasmatic clearance of S (+) ketamine is higher than that of the R (-) ketamine and than that of the S (+) ketamine given in the racemic form (Ihmsen et al., 2001; Henthorn et al., 1999). Another contributing factor is that the 2 isomers compete for the same enzymatic complex in the liver (Kharasch and Labroo, 1992) reducing the rate of its metabolism when administered in the form of the racemate. In the study of Larenza *et al.* (2008) the elimination rate was significantly higher and elimination half-life and mean residence time were lower for S (+) ketamine after S (+)

ketamine compared to racemic ketamine administration. As the action of the dose of S-ketamine tested here was a bit too short in all dogs to allow castration, a constant rate infusion of the drug could follow the initial bolus and like this anaesthesia duration could be extended safely. In the present report the relevant dogs were successfully castrated with a further bolus. Their cardiopulmonary and recovery data however was excluded from the study.

For recovery medetomidine's action was antagonised by its antagonist atipamezole 1 h after the initial ketamine application. At the dose rates of medetomidine tested otherwise recovery would have been delayed. Medetomidine pharmacokinetics in cats and dogs are characterized by a peak concentration after IM administration within 0.5 h. Elimination of medetomidine from plasma/serum occurred with half-lives ranging from 0.97 to 1.60 h. (Salonen,1989). As the dogs recovered thereafter quickly and smoothly it is unlikely, that there was still significant residual action of either ketamine.

Convulsions. Two dogs, one belonging to s-ket group and one belonging to rac-ket group suffered from seizures-like episodes approximately 30 minutes after ketamine administration. Both dogs were treated with benzodiazepines which stopped the seizures immediately. However were these two dogs data excluded from evaluation of post-operative data because they received supplemental medication that influenced the anaesthesia. Convulsions during anaesthesia of dogs have multiple causes (Lervik et al., 2010). Ketamine at higher doses has been associated with convulsions, increased muscle tone and spontaneous muscular activity in dogs (Haskins et al. 1985). Both patient recovered well from anaesthesia and were returned to their respective owner the same day of surgery.

7.5 Recovery

Overall there were no differences between the two groups concerning recovery.

Assessment of behaviour. The behavioural changes after racemic ketamine are well known and have been the reason for reluctance of many practitioners to administer such a drug to their patients (Kohr and Durieux, 1998). Typical ketamine effects on behaviour, are: ataxia, increased motor activity, hyperreflexia, hallucinations (dogs are like "watching butterflies"), head movements, tongue movements and increased saliva production. In previous studies in dogs, the use of S (+) ketamine anaesthesia resulted in the shorter duration of unconsciousness, shorter time to sternal recumbency (TSR) and more quiet recovery from anaesthesia when compared to racemic ketamine (Muir and Hubbell, 1988). The application of a smaller dose of S (+) ketamine, in comparison with racemic ketamine, produced a faster recovery from anaesthesia with a minor incidence of psychomimetic side effects, at similar cardiovascular effects during anaesthesia (Oleskovicz *et al.*, 2009; de Almeida *et al.*, 2005; Duque *et al.*, 2004). In the present study all dogs were sedated with medetomidine. There were no statistical differences in regard to time to first movement ($p = 0.09$), time to sternal position ($p =$

0.36) and time to sitting position (p = 0.94) between both groups. In contrast with previous studies in cats and dogs, in the present study no statistical differences were found. Differences between racemic ketamine and S (+) ketamine were probably masked by medetomidine's strong sedative action. Following its reversal, 60 minutes after the application of the ketamine, sedation and behaviour scores at all time points were similar between both groups, as the effects of either ketamine had vanished by then.

Diarrhoea. One dog, 60 minutes after atipamezole administration, showed once a diarrhoea episode. There are multiple causes for diarrhoea episodes during the post-operative phase in dogs including stress, NSAID and concomitant not reported disease. Because the animal shelter's keepers reported no new episodes of diarrhoea in the following 5 days, a concomitant disease vs. cause can be excluded.

The peri-operative procedure includes many possible stressors (dogs belonging to the animal shelter, environmental factors at the clinic and recovery quality). The importance of the different individual responses is not evaluable, in fact pre-operative stress effects during the whole peri-operative period and especially the post-anaesthetic recovery phase are still understudied (Väisänen *et al.*, 2005)

NSAIDs can provoque an acute as well as a chronic toxicosis. Toxicosis are usually manifested by gastrointestinal upset including: nausea, vomiting, haemorrhage and ulceration (Raekallio *et al.* 2006; Vollmar, 1993). Dogs are especially sensitive to those drugs, and reports of serious, and occasionally fatal, complications are numerous. Carprofen is a propionic acid-derived NSAID that has anti-inflammatory, analgesic, and antipyretic activity. In animals, carprofen is as potent as indomethacin and more potent than aspirin or phenylbutazone, but appears to be safer than most other NSAIDs (Fox *et al.*, 1997; Papich, 1997). In a study on effects of short-term sequential administration of NSAID drugs (carprofen and deracoxib) on the stomach and proximal portion of the duodenum in healthy dogs, lesion worsening from day before were observed after day 2. But none of the tested drugs did cause clinically important gastro-duodenal ulcers (Dowers et al., 2006). In our study all dogs were treated with a single injection of carprofen at the dose of 4 mg/kg BW immediately after the end of surgery. Therefore it is unlikely that carprofen alone caused important lesions or symptoms but in combination with stress it might help to cause diarrhoea.

8. Conclusion

The main goal of the present study was to compare safety and effectiveness of anaesthesia (induction, maintenance and recovery qualities) in dogs undergoing elective neutering surgery, anaesthetised with racemic ketamine and with S (+) ketamine, at half the dose rate of the racemic ketamine. At these doses, both groups showed a comparable degree of intra-operative anaesthesia. The fact, that two dogs in the S (+) ketamine needed re-dosing might indicate, that duration of action of S (+) ketamine at the tested dose rates is slightly shorter than the one of the racemic ketamine, as shown for other species before. In compromised patients and older patients this is certainly an advantage of S (+) ketamine over racemic ketamine. At the tested dose ranges respiration was better maintained with S (+) ketamine, which in practice is another important factor to increase safety.

Taking all together, it is concluded that male dogs undergoing routine neutering surgery and anaesthetised with a single dose of intravenous S (+) ketamine showed acceptable intra-operative anaesthetic conditions with post-anaesthetic parameters within the physiological range similar to those of racemic ketamine by the use of only 50% of the dose. S (+) ketamine has been demonstrated to be safe and effective for induction, maintenance and recovery of short anaesthesia in dogs sedated with medetomidine undergoing surgical castration.

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Annexes

Annex A: Owner consent (Conséntement du propriétaire)

Consentement du propriétaire

Chère Madame, Cher Monsieur,

Vous avez confié votre chien pour une orchiectomie élective au cabinet vétérinaire de La Confrérie, à Epalinges. Pour cette intervention chirurgicale, il est prévu, comme de routine, de soumettre le chien à une anesthésie générale. Sur les risques d'une anesthésie.

Notre protocole d' anesthésie de routine comprend trois phases : une mise en sédation, une induction (si nécessaire une maintenance de la narcose) et une phase de réveil.
La sédation est composé de deux produits (Domitor et Morphasol) afin d'obtenir une analgésie et sédation optimales pour le chien.
L'induction à l' anesthésie est garantie à l'aide de Keta-S, substance qui permet aussi de maintenir aussi l'anesthésie pendant toute la durée de l'intervention (orchiectomie).

Le principe actif utilisé, la kétamine-S, est une nouvelle forme (isomère) qui a évoluée de la kétamine (racémique), substance qui est très apprécié dans la médecine vétérinaire depuis déjà des années.
L'expérience faite avec la kétamine, a montré, malgré une efficacité très bonne, des effets secondaires pas bénéfiques pour les animaux. La kétamine-S en revanche, diminue ces effets secondaires et permet à l'animal soumis à une anesthésie générale, de vivre une phase de réveil plus douce et moins traumatisante.
Les effets positifs de la kétamine-S ont déjà été démontrés dans le cadre d'études cliniques sur l'être humain et les animaux.
Aujourd'hui, la kétamine-S est déjà sur le marché avec le nom Keta-S. Il est dans nos intentions de laisser bénéficier aussi les chiens de la kétamine-S. Pour enregistrer le produit au prés de Swissmedic, j' ai reçu le mandat de réaliser cette étude.
C'est avec cette nouvelle et performante forme de kétamine (la kétamine–S) que votre chien sera soumis à une anesthésie générale pour sa castration (orchiectomie). Pendant toute la durée de l'intervention, les effets cliniques de cette dernière seront surveillés, saisis, analysés pour enfin être comparés avec les effets de sa forme plus ancienne, la kétamine racémique.
Pour pouvoir accepter votre chien dans cette étude, il me faut votre accord sous forme de signature.

Je suis reconnaissant de recevoir votre soutien dans la réalisation de ce projet et vous remercie. La castration des chiens qui font partie désormais de cette étude ne vous sera pas facturé. En cas de questions, je reste volontiers à votre disposition.

med. vet. Boris Pfaender
La Confrérie, Cabinet vétérinaire
1066 Epalinges

Nom du Propriétaire : _____

Nom du chien, Age, Race : _____

_____

_____

_____

Lieu, Date, Signature : ----------

Annex B: Case Reports Forms (CRF)

CRF page 1 of 4

Patient data
Name:
Owner:
Breed:
Age:

CTKet6VD07: Pre-admission Examination

CRF N.....

Date: ___/____/___

Group A B

ASA I II III IV V

Pre-anesthesia exam

Body condition

Weight: obesity - cachexia - normal
Hydratation status: T°C:

Cardiovascular and respiratory system

Heart rate: Pulse character:
Respiratory rate: Thoracic auscultation:
Mucous membrane colour: Capillary refill time:

Inclusion Yes No

Venous catheter right left
(V. brachiocephalica)

Sedation

Time	Drug	Dose (mg/kg)	Total (mg)	Total (ml)	Observations
	Medetomidine (Dorbene®) (1 mg/ml)	0.450 mg/m²			
	Butorphanol-10 (Morphasol) (10 mg/ml)	0.2 mg/kg			

Heart rate: Pulse character: Respiratory rate:
Mucous membrane colour: Capillary refill time:
Sedation time:

Anaesthesia induction

Time	Drug	Dose (ml)	Observations
	Group A or B		

Intubation Time: Tubus:

_____ date

_____
Signature

CTKet6VD07: Anaesthesia Monitoring

CRF N.....

Time								**Observations**
O_2 Flow (L/min)								
O_2 FI %								
End tidal CO_2 (mm Hg)								
Heart rate								
Blood pressure	Systolic							
	Mean							
	Diastolic							
Respiratory rate								
SpO_2 %								
Mucous membrane colour								
Capillary refill time								
Eyelid reflex (+/-)								
Body position								
Various								

***S*: Surgery beginning** ***E*: surgery ending**

Lidocaine injection: dose: mg= ml

Carprofen injection: dose: mg = ml Amoxicillin/clavulanic injection: dose: mg= ml

Ketamine redosing: Yes , dose (ml): No

Extubation time:

_____ _____

date

signature

CTKet6VD07: Recovery 1

CRF N.....

Atipamezole (Antisedan®) injection:dose:_____ ml

Atipamezol injection time:____

Clinical data	T 0	T 30	T 60
Heart rate			
Pulse character			
Capillary refill time			
Respiratory rate			
Mucous membrane colour			
Temperature			

T = timepoint

_____ _____

date signature

Timepoints	T0	T10	T 20	T 30	T 60
Sedation score					
0 = Non sedation (fully alert, reacting to noises)					
1 = Poor sedation (alert but somnolent, reacting to noises)					
2 = Moderate sedation (drowsy, but occasionally restless)					
3 = Deep sedation (sleeping comfortable)					
Behavioural Score					
0 = Normal behaviour pattern (quiet, attentive)					
1 = Minor changes (turns head, minor ataxia, minor excitation)					
2 = Moderate changes (moderate ataxia, moderate excitation)					
3 = Severe changes (restless, severe ataxia, severe excitation)					
Vocalizations					
0 = No vocalizations					
1 = vocalizations					
Muscles					
0 = Relaxation					
1 = Tone increased					
2= Stiffness					
Tremors					
Eyes					
Miosis					
Mydriasis					
Eyes rolled ventrally					
Nystagmus					
Tongue					
0 = no movements					
1 = movements					
Salivation					
0= No salivation					
1 = Salivation					
Re-sedation: No Yes, _____					

CTKet6VD07: Recovery 2 **CRF N.....**

_____ _____

date signature

Annex C: Others drugs used in both groups

Medetomidine

Medetomidine: dose 450 μg/m^2 BSA (body surface area), used intramuscularly (IM), trade name Dorbene® ad us. vet., Injektionslösung Dr. E. Graeub AG, Bern, Switzerland

Butorphanol

Butorphanol: dose 0.2 mg/kg BW, used intramuscularly (IM), trade name Morphasol-10® ad us. vet., Injektionslösung Dr. E. Graeub AG, Bern, Switzerland

Atipamezole

Atipamezole: dose case by case; 2250 μg/m^2 BSA; used intramuscularly (IM); trade name Antisedan® ad us. vet., Injektionslösung; Pfizer AG, Zürich, Switzerland

Carprofen

Carprofen: dose 4 mg/kg BW, used intravenously (IV); trade name Rimadyl® ad us. vet., Injektionslösung; Pfizer AG, Zürich, Switzerland

Amoxicillin / clavulanate

Amoxicillin and clavulanate: dose 8.75 mg/kg BW, used subcutanously (SC); trade name Synulox Suspension®, ad us. vet., Injektionssuspension Pfizer AG, Zürich, Switzerland

Lidocaine

Lidocaine: dose 1 mg/kg BW, trade name Lidocain 2%®; Chassot ad us. vet., Injektionslösung Vetoquinol AG, Switzerland